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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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TITLE OF THE INVENTION (500 characters max)				
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<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27.				
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[Page 1 of 2]

Respectfully submitted,

Date April 1, 2004

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USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

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METHODS FOR IDENTIFYING RISK OF OSTEOARTHRITIS AND TREATMENTS THEREOF

Field of the Invention

[0001] The invention relates to genetic methods for identifying risk of osteoarthritis and treatments that specifically target such diseases.

Background

[0002] Osteoarthritis (OA) is a chronic disease usually affecting weight-bearing synovial joints. There are approximately 20 million Americans affected by OA and it is the leading cause of disability in the United States. In addition to extensive human suffering, OA also accounts for nearly all knee replacements and more than half of all hip replacements in the United States. Despite its prevalence, OA is poorly understood and there are few treatments available besides anti-inflammatory drugs and joint replacement.

[0003] Most commonly affecting middle-aged and older people, OA can range from very mild to very severe. It affects hands and weight-bearing joints such as knees, hips, feet and the back. Knee OA can be as disabling as any cardiovascular disease except stroke.

[0004] OA is characterized by the breakdown of cartilage in joints. Cartilage in joints cushions the ends of bones, and cartilage breakdown causes bones to rub against each other, causing pain and loss of movement. Type II collagen is the main component of cartilage, comprising 15-25% of the wet weight, approximately half the dry weight, and representing 90-95% of the total collagen content in the tissue. It forms fibrils that endow cartilage with tensile strength (Mayne, R. Arthritis Rheum. 32:241-246 (1989)).

Summary

[0005] It has been discovered that certain polymorphic variations in human genomic DNA are associated with osteoarthritis. In particular, polymorphic variants in a locus containing *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* and *GPR50* regions in human genomic DNA have been associated with risk of osteoarthritis.

[0006] Thus, featured herein are methods for identifying a subject at risk of osteoarthritis and/or a risk of osteoarthritis in a subject, which comprise detecting the presence or absence of one or more polymorphic variations associated with osteoarthritis in or around the loci described herein in a human nucleic acid sample. In an embodiment, two or more polymorphic variations are detected in two or more regions of which one is the *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50*

region. In certain embodiments, 3 or more, or 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 or more polymorphic variants are detected.

[0007] Also featured are nucleic acids that include one or more polymorphic variations associated with occurrence of osteoarthritis, as well as polypeptides encoded by these nucleic acids. In addition, provided are methods for identifying candidate therapeutic molecules for treating osteoarthritis, as well as methods for treating osteoarthritis in a subject by identifying a subject at risk of osteoarthritis and treating the subject with a suitable prophylactic, treatment or therapeutic molecule.

[0008] Also provided are compositions comprising a cell from a subject having osteoarthritis or at risk of osteoarthritis and/or a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleic acid, with a RNAi, siRNA, antisense DNA or RNA, or ribozyme nucleic acid designed from a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleotide sequence. In an embodiment, the RNAi, siRNA, antisense DNA or RNA, or ribozyme nucleic acid is designed from a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleotide sequence that includes one or more polymorphic variations associated with osteoarthritis, and in some instances, specifically interacts with such a nucleotide sequence. Further, provided are arrays of nucleic acids bound to a solid surface, in which one or more nucleic acid molecules of the array have a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleotide sequence, or a fragment or substantially identical nucleic acid thereof, or a complementary nucleic acid of the foregoing. Featured also are compositions comprising a cell from a subject having osteoarthritis or at risk of osteoarthritis and/or a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* polypeptide, with an antibody that specifically binds to the polypeptide. In an embodiment, the antibody specifically binds to an epitope in the polypeptide that includes a non-synonymous amino acid modification associated with osteoarthritis (e.g., results in an amino acid substitution in the encoded polypeptide associated with osteoarthritis). Thus, featured is an antibody that selectively binds to an epitope in a *APOB* polypeptide having an amino acid encoded by a polymorphic site associated with osteoarthritis (e.g., an epitope comprising a threonine or isoleucine encoded by rs1367117 (e.g., a threonine at position 98 in an *APOB* polypeptide)).

Brief Description of the Drawings

[0009] Figures 1A-1F show proximal SNPs in *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *LOXL1* and *CASPR4* regions of genomic DNA, respectively. The position of each SNP in the chromosome is shown on the x-axis and the y-axis provides the negative logarithm of the p-value comparing the estimated allele to that of the control group. Also shown in the figures are exons and introns of the regions in the approximate chromosomal positions.

Detailed Description

[0010] It has been discovered that a polymorphic variant in a locus containing a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXLI*, *CASPR4* or *GPR50* region is associated with occurrence of osteoarthritis in subjects. Thus, detecting genetic determinants associated with an increased risk of osteoarthritis occurrence can lead to early identification of a predisposition to osteoarthritis and early prescription of preventative measures. Also, associating a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXLI*, *CASPR4* or *GPR50* polymorphic variant with osteoarthritis has provided new targets for screening molecules useful in treatments of osteoarthritis.

Osteoarthritis and Sample Selection

[0011] Osteoarthritis (OA), or degenerative joint disease, is one of the oldest and most common types of arthritis. It is characterized by the breakdown of the joint's cartilage. Cartilage is the part of the joint that cushions the ends of bones, and its breakdown causes bones to rub against each other, causing pain and loss of movement. Type II collagen is the main component of cartilage, comprising 15-25% of the wet weight, approximately half the dry weight, and representing 90-95% of the total collagen content in the tissue. It forms fibrils that endow cartilage with tensile strength (Mayne, R. Arthritis Rheum. 32:241-246 (1989)).

[0012] Most commonly affecting middle-aged and older people, OA can range from very mild to very severe. It affects hands and weight-bearing joints such as knees, hips, feet and the back. Knee OA can be as disabling as any cardiovascular disease except stroke.

[0013] Osteoarthritis affects an estimated 20.7 million Americans, mostly after age 45, with women more commonly affected than men. Physicians make a diagnosis of OA based on a physical exam and history of symptoms. X-rays are used to confirm diagnosis. Most people over 60 reflect the disease on X-ray, and about one-third have actual symptoms.

[0014] There are many factors that can cause OA. Obesity may lead to osteoarthritis of the knees. In addition, people with joint injuries due to sports, work-related activity or accidents may be at increased risk of developing OA.

[0015] Genetics has a role in the development of OA. Some people may be born with defective cartilage or with slight defects in the way that joints fit together. As a person ages, these defects may cause early cartilage breakdown in the joint or the inability to repair damaged or deteriorated cartilage in the joint.

[0016] Inclusion or exclusion of samples for an osteoarthritis pool may be based upon the following criteria: ethnicity (e.g., samples derived from an individual characterized as Caucasian); parental

ethnicity (e.g., samples derived from an individual of British paternal and maternal descent); relevant phenotype information for the individual (e.g., case samples derived from individuals diagnosed with specific knee osteoarthritis (OA) and were recruited from an OA knee replacement clinic). Control samples may be selected based on relevant phenotype information for the individual (e.g., derived from individuals free of OA at several sites (knee, hand, hip etc)); and no family history of OA and/or rheumatoid arthritis. Additional phenotype information collected for both cases and controls may include age of the individual, gender, family history of OA, diagnosis with osteoarthritis (joint location of OA, date of primary diagnosis, age of individual as of primary diagnosis), knee history (current symptoms, any major knee injury, menisectomy, knee replacement surgery, age of surgery), HRT history, osteoporosis diagnosis.

[0017] Based in part upon selection criteria set forth above, individuals having osteoarthritis can be selected for genetic studies. Also, individuals having no history of osteoarthritis often are selected for genetic studies, as described hereafter.

Polymorphic Variants Associated with Osteoarthritis

[0018] A genetic analysis provided herein linked osteoarthritis with polymorphic variant nucleic acid sequences in the human genome. As used herein, the term “polymorphic site” refers to a region in a nucleic acid at which two or more alternative nucleotide sequences are observed in a significant number of nucleic acid samples from a population of individuals. A polymorphic site may be a nucleotide sequence of two or more nucleotides, an inserted nucleotide or nucleotide sequence, a deleted nucleotide or nucleotide sequence, or a microsatellite, for example. A polymorphic site that is two or more nucleotides in length may be 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more, 20 or more, 30 or more, 50 or more, 75 or more, 100 or more, 500 or more, or about 1000 nucleotides in length, where all or some of the nucleotide sequences differ within the region. A polymorphic site is often one nucleotide in length, which is referred to herein as a “single nucleotide polymorphism” or a “SNP.”

[0019] Where there are two, three, or four alternative nucleotide sequences at a polymorphic site, each nucleotide sequence is referred to as a “polymorphic variant” or “nucleic acid variant.” Where two polymorphic variants exist, for example, the polymorphic variant represented in a minority of samples from a population is sometimes referred to as a “minor allele” and the polymorphic variant that is more prevalently represented is sometimes referred to as a “major allele.” Many organisms possess a copy of each chromosome (e.g., humans), and those individuals who possess two major alleles or two minor alleles are often referred to as being “homozygous” with respect to the polymorphism, and those individuals who possess one major allele and one minor allele are normally referred to as being “heterozygous” with respect to the polymorphism. Individuals who are homozygous with respect to one

allele are sometimes predisposed to a different phenotype as compared to individuals who are heterozygous or homozygous with respect to another allele.

[0020] In genetic analysis that associate polymorphic variants with osteoarthritis, samples from individuals having osteoarthritis and individuals not having osteoarthritis often are allelotyped and/or genotyped. The term “allelotype” as used herein refers to a process for determining the allele frequency for a polymorphic variant in pooled DNA samples from cases and controls. By pooling DNA from each group, an allele frequency for each SNP in each group is calculated. These allele frequencies are then compared to one another. The term “genotyped” as used herein refers to a process for determining a genotype of one or more individuals, where a “genotype” is a representation of one or more polymorphic variants in a population.

[0021] A genotype or polymorphic variant may be expressed in terms of a “haplotype,” which as used herein refers to two or more polymorphic variants occurring within genomic DNA in a group of individuals within a population. For example, two SNPs may exist within a gene where each SNP position includes a cytosine variation and an adenine variation. Certain individuals in a population may carry one allele (heterozygous) or two alleles (homozygous) having the gene with a cytosine at each SNP position. As the two cytosines corresponding to each SNP in the gene travel together on one or both alleles in these individuals, the individuals can be characterized as having a cytosine/cytosine haplotype with respect to the two SNPs in the gene.

[0022] As used herein, the term “phenotype” refers to a trait which can be compared between individuals, such as presence or absence of a condition, a visually observable difference in appearance between individuals, metabolic variations, physiological variations, variations in the function of biological molecules, and the like. An example of a phenotype is occurrence of osteoarthritis.

[0023] Researchers sometimes report a polymorphic variant in a database without determining whether the variant is represented in a significant fraction of a population. Because a subset of these reported polymorphic variants are not represented in a statistically significant portion of the population, some of them are sequencing errors and/or not biologically relevant. Thus, it is often not known whether a reported polymorphic variant is statistically significant or biologically relevant until the presence of the variant is detected in a population of individuals and the frequency of the variant is determined. Methods for detecting a polymorphic variant in a population are described herein, specifically in Example 2. A polymorphic variant is statistically significant and often biologically relevant if it is represented in 5% or more of a population, sometimes 10% or more, 15% or more, or 20% or more of a population, and often 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, or 50% or more of a population.

[0024] A polymorphic variant may be detected on either or both strands of a double-stranded nucleic acid. Also, a polymorphic variant may be located within an intron or exon of a gene or within a portion

of a regulatory region such as a promoter, a 5' untranslated region (UTR), a 3' UTR, and in DNA (*e.g.*, genomic DNA (gDNA) and complementary DNA (cDNA)), RNA (*e.g.*, mRNA, tRNA, and rRNA), or a polypeptide. Polymorphic variations may or may not result in detectable differences in gene expression, polypeptide structure, or polypeptide function.

[0025] It was determined that polymorphic variations associated with an increased risk of osteoarthritis existed in *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXLI*, *CASPR4* and *GPR50* regions. In certain embodiments, polymorphic variants at positions rs910223, rs1367117, rs1024791, rs1465621, rs1018810, rs242392, rs8818, rs1395486 and rs512294 in the human genome were associated with an increased risk of osteoarthritis, and in specific embodiments, an adenine at position rs910223, a guanine at position rs1367117, a guanine at position rs1024791, an adenine at position rs1465621, an adenine at position rs1018810, a thymine at position rs242392, a cytosine at position rs8818, a thymine at position rs1395486 and a guanine at position rs512294 were associated with an increased risk of osteoarthritis.

[0026] Polymorphic variants in and around the *APOB* locus were tested for association with osteoarthritis. These include polymorphic variants at positions in SEQ ID NO: 1 selected from the group consisting of 238, 294, 295, 347, 1425, 4891, 5087, 7041, 7121, 7219, 7443, 7485, 10939, 11367, 11571, 11839, 12551, 12646, 13469, 14913, 15205, 15246, 15695, 17473, 17610, 17828, 18130, 18281, 18623, 18890, 21561, 23100, 23872, 24581, 24582, 24983, 27540, 30846, 31415, 31453, 31899, 37000, 38681, 39287, 42951, 45648, 46222, 46687, 47020, 47593, 48513, 49723, 49986, 53018, 53296, 53547, 53899, 53916, 53933, 54305, 55327, 55895, 56143, 56640, 58486, 59576, 63048, 64008, 64018, 64859, 65995, 66905, 67183, 67942, 68101, 68521, 68664, 68988, 69178, 72143, 74183, 74312, 74407, 75518, 76153, 77398, 77615, 79092, 80000, 80125, 80595, 81061, 81151, 81918, 83072, 83137, 83235, 83263, 83279, 83280, 83533, 86856, 87186, 87189, 87727, 87978, 89129, 89556, 89702, 90233, 93060, 94779, 95367, 95844, 95942, 96884, 96938, 97627, 97777, 97871, 98746 and 99663. Polymorphic variants at the following positions in SEQ ID NO: 1 in particular were associated with an increased risk of osteoarthritis: 7219, 7485, 11839, 31899, 37000, 48513, 49986, 56640, 74407, 77398, 93060 and 97627. In particular, the following polymorphic variants in SEQ ID NO: 1 were associated with risk of osteoarthritis: an adenine at position 7219, a guanine at position 7485, an adenine at position 11839, a thymine at position 31899, an adenine at position 37000, a cytosine at position 48513, a guanine at position 49986, a guanine at position 56640, a cytosine at position 74407, a guanine at position 77398, an adenine at position 93060 and an adenine at position 97627. A threonine at amino acid position 98 in an *APOB* polypeptide was associated with increased risk of osteoarthritis (*i.e.*, an isoleucine to threonine non-synonymous variation).

[0027] Polymorphic variants in and around the *IL1RL2* locus were tested for association with osteoarthritis. These include polymorphic variants at positions in SEQ ID NO: 2 selected from the group consisting of 225, 509, 860, 874, 939, 1483, 1798, 2189, 2215, 2282, 2340, 2963, 3369, 3481, 3564, 3653, 4860, 4941, 4975, 5321, 5346, 5541, 5633, 6007, 6317, 6378, 6382, 6426, 6479, 6641, 6703, 6705, 7963, 8525, 8526, 8598, 8624, 8883, 8980, 13578, 16135, 16141, 16642, 16931, 17004, 17009, 17010, 18713, 18853, 20783, 21335, 22180, 22268, 22285, 25378, 25906, 26015, 26475, 26798, 27042, 27649, 27827, 27873, 28122, 28202, 28232, 28240, 29546, 29748, 30054, 30646, 31149, 36912, 36936, 37184, 39064, 39343, 40868, 40917, 41113, 47343, 47806, 47911, 48009, 48621, 49245, 49247, 49299, 49302, 49514, 49626, 49791, 50010, 50294, 51482, 51556, 51855, 51956, 52155, 52448, 52458, 52511, 52607, 54049, 54224, 54567, 55052, 55857, 55941, 56120, 56349, 56727, 57232, 58806, 61181, 63808, 64526, 64865, 64928, 64966, 65080, 65690, 66228, 66982, 72511, 74170, 74264, 74333, 74502, 74741, 75321, 82558, 85366, 85469, 86485, 87687, 89463, 89660, 95718 and 95821. Polymorphic variants at the following positions in SEQ ID NO: 2 in particular were associated with an increased risk of osteoarthritis: 2215, 3369, 16642, 20783, 52155, 55052, 55941, 74333, 74741, 85366, 85469, 87687, 89660 and 95718, where specific embodiments are directed to position 52155. In particular, the following polymorphic variants in SEQ ID NO: 2 were associated with risk of osteoarthritis: an adenine at position 2215, a deletion at position 3369, a deletion at position 16642, a cytosine at position 20783, a cytosine at position 52155, a cytosine at position 55052, a cytosine at position 55941, a thymine at position 74333, an adenine at position 74741, a deletion at position 85366, a thymine at position 85469, a thymine at position 87687, an adenine at position 89660 and a cytosine at position 95718.

[0028] Polymorphic variants in and around the *WASPIP* locus were tested for association with osteoarthritis. These include polymorphic variants at positions in SEQ ID NO: 3 selected from the group consisting of 209, 5908, 7460, 7733, 7855, 7904, 8869, 9480, 13820, 15152, 17713, 17804, 18220, 19083, 19123, 19605, 20247, 20592, 21907, 23273, 23299, 23623, 23669, 23844, 24190, 24486, 24896, 25118, 30551, 30844, 30900, 30942, 31699, 32081, 35078, 36196, 36541, 38356, 45578, 49634, 49774, 51119, 51181, 51652, 54467, 55762, 55999, 57865, 66613, 68377, 69754, 72859, 76512, 76717, 77722, 80998, 82033, 89658, 89960, 94155 and 95679. Polymorphic variants at the following positions in SEQ ID NO: 3 in particular were associated with an increased risk of osteoarthritis: 19083, 30900, 38356, 76512 and 94155, where specific embodiments are directed to positions 30900, 76512 and/or 94155. In particular, the following polymorphic variants in SEQ ID NO: 3 were associated with risk of osteoarthritis: a thymine at position 19083, a guanine at position 30900, an adenine at position 38356, an adenine at position 76512 and an adenine at position 94155.

[0029] Polymorphic variants in and around the *BVES* locus were tested for association with osteoarthritis. These include polymorphic variants at positions in SEQ ID NO: 4 selected from the group

consisting of 241, 801, 899, 2091, 2290, 2440, 4959, 7914, 7969, 7972, 10831, 12399, 13841, 14461, 14680, 16808, 18231, 18394, 18505, 18684, 19257, 20263, 20656, 21499, 21563, 21612, 21834, 22406, 22408, 22685, 23303, 23306, 25139, 25211, 25364, 25381, 25414, 25835, 26214, 27224, 27526, 27934, 28550, 29015, 29879, 29979, 30030, 30585, 31753, 31934, 33227, 33228, 35172, 36901, 36921, 36932, 37061, 37570, 38745, 38970, 39725, 40070, 40460, 41470, 41562, 41956, 42047, 42280, 42358, 42629, 43075, 43387, 43393, 43438, 44115, 44537, 45642, 46629, 47496, 47515, 48329, 48862, 48908, 49038, 49080, 50204, 50404, 50426, 50531, 50840, 50964, 50971, 51378, 52610, 53906, 53951, 54111, 54149, 55563, 55999, 58415, 58961, 60447, 61377, 61528, 61606, 62140, 62461, 63826, 64950, 65076, 66121, 66406, 67051, 68860, 69014, 70796, 72325, 73414, 75258, 76347, 76839, 77358, 77822, 77946, 80002, 80024, 80285, 80397, 82075, 82153, 83981, 84184, 85089, 85288, 85330, 85581, 85642, 86433, 86904, 88391, 89042, 90828, 92676, 92881, 94227, 94585, 94616, 94712, 94738, 95253, 95522, 95869 and 97856. Polymorphic variants at the following positions in SEQ ID NO: 4 in particular were associated with an increased risk of osteoarthritis: 25414, 25835, 38970, 41470, 44115, 47496, 49038, 50204, 50840, 50964, 50971, 53906, 54149, 58415, 70796, 72325, 75258, 77822, 80002, 85288, 85581, 86904, 90828, 94616, 94712, 95869 and 97856. In particular, the following polymorphic variants in SEQ ID NO: 4 were associated with risk of osteoarthritis: an adenine at position 25414, a cytosine at position 25835, an adenine at position 38970, an adenine at position 41470, an adenine at position 44115, a guanine at position 47496, a cytosine at position 49038, an adenine at position 50204, a thymine at position 50840, a cytosine at position 50964, a cytosine at position 50971, an adenine at position 53906, a guanine at position 54149, a guanine at position 58415, a thymine at position 70796, a guanine at position 72325, a cytosine at position 75258, an adenine at position 77822, an adenine at position 80002, an adenine at position 85288, an adenine at position 85581, a guanine at position 86904, a guanine at position 90828, an adenine thymine adenine adenine sequence at position 94616, a cytosine at position 94712, a guanine at position 95869 and a cytosine at position 97856.

[0030] Polymorphic variants in and around the *LOXLI* locus were tested for association with osteoarthritis. These include polymorphic variants at positions in SEQ ID NO: 5 selected from the group consisting of 213, 249, 1824, 2057, 2306, 2869, 3976, 4288, 4290, 4434, 5298, 5467, 8486, 8487, 8831, 9036, 9058, 9131, 9732, 9862, 10191, 10270, 16167, 17620, 17751, 17764, 17787, 19401, 21021, 21902, 22173, 22416, 22653, 24945, 25011, 28563, 48574, 48710, 48880, 50194, 56343, 56455, 56729, 56759, 56895, 57036, 57702, 62515, 62629, 63501, 63547, 64876, 65073, 67149, 67549, 71660, 71906 and 71911. A polymorphic variant at position 65073 in SEQ ID NO: 5, often a guanine, in particular was associated with an increased risk of osteoarthritis.

[0031] Polymorphic variants in and around the *CASPR4* locus were tested for association with osteoarthritis. These include polymorphic variants at positions in SEQ ID NO: 6 selected from the group

consisting of 205, 866, 4212, 5934, 11486, 16969, 22509, 22796, 28097, 28626, 28853, 28873, 30155, 30827, 31956, 32404, 32944, 35205, 35227, 35781, 41052, 45051, 46039, 47276, 47678, 47716, 51014, 54408, 54596, 56853, 61851, 62016, 62461, 68257, 69793, 73976, 73999, 74053, 75315, 75729, 76466, 77216, 77217, 79239, 80825, 81060, 81097, 81426, 84787, 84896, 85165, 86502, 86753, 86941, 88787 and 95598. Polymorphic variants at the following positions in SEQ ID NO: 6 in particular were associated with an increased risk of osteoarthritis: 47716 and 69793. In particular, the following polymorphic variants in SEQ ID NO: 6 were associated with risk of osteoarthritis: an adenine at position 47716 and a thymine at position 69793.

[0032] Based in part upon analyses summarized in Figures 1A-1F, a region with significant association has been identified in a locus associated with osteoarthritis. Any polymorphic variants associated with osteoarthritis in a region of significant association can be utilized for embodiments described herein. For example, polymorphic variants in a region spanning positions 21233000 to 21243000 (approximately 10,000 nucleotides in length) in a *APOB* locus, a region spanning chromosome positions 102456500 to 102471500 (approximately 15,000 nucleotides in length) in a *IL1RL2* locus, a region spanning chromosome positions 175647734 to 175655734 (approximately 8,000 nucleotides in length) in a *WASPIP* locus, a region spanning chromosome positions 105595000 to 105615000 (approximately 20,000 nucleotides in length) in a *BVES* locus, a region spanning chromosome positions 71957600 to 71962600 (approximately 5,000 nucleotides in length) in a *LOXLI* locus and a region spanning chromosome positions 76221000 to 76226000 (approximately 5,000 nucleotides in length) in a *CASPR4* locus, have significant association (chromosome positions are within NCBI's Genome build 34).

Additional Polymorphic Variants Associated with Osteoarthritis

[0033] Also provided is a method for identifying polymorphic variants proximal to an incident, founder polymorphic variant associated with osteoarthritis. Thus, featured herein are methods for identifying a polymorphic variation associated with osteoarthritis that is proximal to an incident polymorphic variation associated with osteoarthritis, which comprises identifying a polymorphic variant proximal to the incident polymorphic variant associated with osteoarthritis, where the incident polymorphic variant is in a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXLI*, *CASPR4* or *GPR50* nucleotide sequence. The nucleotide sequence often comprises a polynucleotide sequence selected from the group consisting of (a) a polynucleotide sequence of SEQ ID NO: 1-17; (b) a polynucleotide sequence that encodes a polypeptide having an amino acid sequence encoded by a polynucleotide sequence of SEQ ID NO: 1-17; and (c) a polynucleotide sequence that encodes a polypeptide having an amino acid sequence that is 90% or more identical to an amino acid sequence encoded by a nucleotide

sequence of SEQ ID NO: 1-17 or a polynucleotide sequence 90% or more identical to the polynucleotide sequence of SEQ ID NO: 1-17. The presence or absence of an association of the proximal polymorphic variant with osteoarthritis then is determined using a known association method, such as a method described in the Examples hereafter. In an embodiment, the incident polymorphic variant is a polymorphic variant associated with osteoarthritis described herein. In another embodiment, the proximal polymorphic variant identified sometimes is a publicly disclosed polymorphic variant, which for example, sometimes is published in a publicly available database. In other embodiments, the polymorphic variant identified is not publicly disclosed and is discovered using a known method, including, but not limited to, sequencing a region surrounding the incident polymorphic variant in a group of nucleic samples. Thus, multiple polymorphic variants proximal to an incident polymorphic variant are associated with osteoarthritis using this method.

[0034] The proximal polymorphic variant often is identified in a region surrounding the incident polymorphic variant. In certain embodiments, this surrounding region is about 50 kb flanking the first polymorphic variant (*e.g.* about 50 kb 5' of the first polymorphic variant and about 50 kb 3' of the first polymorphic variant), and the region sometimes is composed of shorter flanking sequences, such as flanking sequences of about 40 kb, about 30 kb, about 25 kb, about 20 kb, about 15 kb, about 10 kb, about 7 kb, about 5 kb, or about 2 kb 5' and 3' of the incident polymorphic variant. In other embodiments, the region is composed of longer flanking sequences, such as flanking sequences of about 55 kb, about 60 kb, about 65 kb, about 70 kb, about 75 kb, about 80 kb, about 85 kb, about 90 kb, about 95 kb, or about 100 kb 5' and 3' of the incident polymorphic variant.

[0035] In certain embodiments, polymorphic variants associated with osteoarthritis are identified iteratively. For example, a first proximal polymorphic variant is associated with osteoarthritis using the methods described above and then another polymorphic variant proximal to the first proximal polymorphic variant is identified (*e.g.*, publicly disclosed or discovered) and the presence or absence of an association of one or more other polymorphic variants proximal to the first proximal polymorphic variant with osteoarthritis is determined.

[0036] The methods described herein are useful for identifying or discovering additional polymorphic variants that may be used to further characterize a gene, region or loci associated with a condition, a disease (*e.g.*, osteoarthritis), or a disorder. For example, allelotyping or genotyping data from the additional polymorphic variants may be used to identify a functional mutation or a region of linkage disequilibrium. In certain embodiments, polymorphic variants identified or discovered within a region comprising the first polymorphic variant associated with osteoarthritis are genotyped using the genetic methods and sample selection techniques described herein, and it can be determined whether those polymorphic variants are in linkage disequilibrium with the first polymorphic variant. The size of

the region in linkage disequilibrium with the first polymorphic variant also can be assessed using these genotyping methods. Thus, provided herein are methods for determining whether a polymorphic variant is in linkage disequilibrium with a first polymorphic variant associated with osteoarthritis, and such information can be used in prognosis/diagnosis methods described herein.

Isolated Nucleic Acids

[0037] Featured herein are isolated *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXLI*, *CASPR4* or *GPR50* nucleic acid variants depicted in SEQ ID NO: 1-17, and substantially identical nucleic acids thereof. A nucleic acid variant may be represented on one or both strands in a double-stranded nucleic acid or on one chromosomal complement (heterozygous) or both chromosomal complements (homozygous).

[0038] As used herein, the term “nucleic acid” includes DNA molecules (*e.g.*, a complementary DNA (cDNA) and genomic DNA (gDNA)) and RNA molecules (*e.g.*, mRNA, rRNA, siRNA and tRNA) and analogs of DNA or RNA, for example, by use of nucleotide analogs. The nucleic acid molecule can be single-stranded and it is often double-stranded. The term “isolated or purified nucleic acid” refers to nucleic acids that are separated from other nucleic acids present in the natural source of the nucleic acid. For example, with regard to genomic DNA, the term “isolated” includes nucleic acids which are separated from the chromosome with which the genomic DNA is naturally associated. An “isolated” nucleic acid is often free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5’ and/or 3’ ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated nucleic acid molecule can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of 5’ and/or 3’ nucleotide sequences which flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an “isolated” nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. As used herein, the term “gene” refers to a nucleotide sequence that encodes a polypeptide.

[0039] Also included herein are nucleic acid fragments. These fragments often have a nucleotide sequence identical to a nucleotide sequence of SEQ ID NO: 1-17, a nucleotide sequence substantially identical to a nucleotide sequence of SEQ ID NO: 1-17, or a nucleotide sequence that is complementary to the foregoing. The nucleic acid fragment may be identical, substantially identical or homologous to a nucleotide sequence in an exon or an intron in a nucleotide sequence of SEQ ID NO: 1-17, and may encode a domain or part of a domain of a polypeptide. Sometimes, the fragment will comprises one or more of the polymorphic variations described herein as being associated with osteoarthritis. The nucleic

acid fragment is often 50, 100, or 200 or fewer base pairs in length, and is sometimes about 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 2000, 3000, 4000, 5000, 10000, 15000, or 20000 base pairs in length. A nucleic acid fragment that is complementary to a nucleotide sequence identical or substantially identical to a nucleotide sequence in SEQ ID NO: 1-17 and hybridizes to such a nucleotide sequence under stringent conditions is often referred to as a “probe.” Nucleic acid fragments often include one or more polymorphic sites, or sometimes have an end that is adjacent to a polymorphic site as described hereafter.

[0040] An example of a nucleic acid fragment is an oligonucleotide. As used herein, the term “oligonucleotide” refers to a nucleic acid comprising about 8 to about 50 covalently linked nucleotides, often comprising from about 8 to about 35 nucleotides, and more often from about 10 to about 25 nucleotides. The backbone and nucleotides within an oligonucleotide may be the same as those of naturally occurring nucleic acids, or analogs or derivatives of naturally occurring nucleic acids, provided that oligonucleotides having such analogs or derivatives retain the ability to hybridize specifically to a nucleic acid comprising a targeted polymorphism. Oligonucleotides described herein may be used as hybridization probes or as components of prognostic or diagnostic assays, for example, as described herein.

[0041] Oligonucleotides are typically synthesized using standard methods and equipment, such as the ABI™3900 High Throughput DNA Synthesizer and the EXPEDITE™ 8909 Nucleic Acid Synthesizer, both of which are available from Applied Biosystems (Foster City, CA). Analogs and derivatives are exemplified in U.S. Pat. Nos. 4,469,863; 5,536,821; 5,541,306; 5,637,683; 5,637,684; 5,700,922; 5,717,083; 5,719,262; 5,739,308; 5,773,601; 5,886,165; 5,929,226; 5,977,296; 6,140,482; WO 00/56746; WO 01/14398, and related publications. Methods for synthesizing oligonucleotides comprising such analogs or derivatives are disclosed, for example, in the patent publications cited above and in U.S. Pat. Nos. 5,614,622; 5,739,314; 5,955,599; 5,962,674; 6,117,992; in WO 00/75372; and in related publications.

[0042] Oligonucleotides may also be linked to a second moiety. The second moiety may be an additional nucleotide sequence such as a tail sequence (*e.g.*, a polyadenosine tail), an adapter sequence (*e.g.*, phage M13 universal tail sequence), and others. Alternatively, the second moiety may be a non-nucleotide moiety such as a moiety which facilitates linkage to a solid support or a label to facilitate detection of the oligonucleotide. Such labels include, without limitation, a radioactive label, a fluorescent label, a chemiluminescent label, a paramagnetic label, and the like. The second moiety may be attached to any position of the oligonucleotide, provided the oligonucleotide can hybridize to the nucleic acid comprising the polymorphism.

Uses for Nucleic Acid Sequence

[0043] Nucleic acid coding sequences may be used for diagnostic purposes for detection and control of polypeptide expression. Also, included herein are oligonucleotide sequences such as antisense RNA, small-interfering RNA (siRNA) and DNA molecules and ribozymes that function to inhibit translation of a polypeptide. Antisense techniques and RNA interference techniques are known in the art and are described herein.

[0044] Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. For example, hammerhead motif ribozyme molecules may be engineered that specifically and efficiently catalyze endonucleolytic cleavage of RNA sequences corresponding to or complementary to *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXLI*, *CASPR4* or *GPR50* nucleotide sequences. Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences, GUA, GUU and GUC. Once identified, short RNA sequences of between fifteen (15) and twenty (20) ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for predicted structural features such as secondary structure that may render the oligonucleotide sequence unsuitable. The suitability of candidate targets may also be evaluated by testing their accessibility to hybridization with complementary oligonucleotides, using ribonuclease protection assays.

[0045] Antisense RNA and DNA molecules, siRNA and ribozymes may be prepared by any method known in the art for the synthesis of RNA molecules. These include techniques for chemically synthesizing oligodeoxyribonucleotides well known in the art such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors which incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly, depending on the promoter used, can be introduced stably into cell lines.

[0046] DNA encoding a polypeptide also may have a number of uses for the diagnosis of diseases, including osteoarthritis, resulting from aberrant expression of a target gene described herein. For example, the nucleic acid sequence may be used in hybridization assays of biopsies or autopsies to diagnose abnormalities of expression or function (*e.g.*, Southern or Northern blot analysis, *in situ* hybridization assays).

[0047] In addition, the expression of a polypeptide during embryonic development may also be determined using nucleic acid encoding the polypeptide. As addressed, *infra*, production of functionally

impaired polypeptide is the cause of various disease states, such as osteoarthritis. *In situ* hybridizations using polypeptide as a probe may be employed to predict problems related to osteoarthritis. Further, as indicated, *infra*, administration of human active polypeptide, recombinantly produced as described herein, may be used to treat disease states related to functionally impaired polypeptide. Alternatively, gene therapy approaches may be employed to remedy deficiencies of functional polypeptide or to replace or compete with dysfunctional polypeptide.

Expression Vectors, Host Cells, and Genetically Engineered Cells

[0048] Provided herein are nucleic acid vectors, often expression vectors, which contain a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXLI*, *CASPR4* or *GPR50* nucleotide sequence, or a substantially identical sequence thereof. As used herein, the term “vector” refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked and can include a plasmid, cosmid, or viral vector. The vector can be capable of autonomous replication or it can integrate into a host DNA. Viral vectors may include replication defective retroviruses, adenoviruses and adeno-associated viruses for example.

[0049] A vector can include a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXLI*, *CASPR4* or *GPR50* nucleotide sequence in a form suitable for expression of an encoded target polypeptide or target nucleic acid in a host cell. A “target polypeptide” is a polypeptide encoded by a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXLI*, *CASPR4* or *GPR50* nucleotide sequence, or a substantially identical nucleotide sequence thereof. The recombinant expression vector typically includes one or more regulatory sequences operatively linked to the nucleic acid sequence to be expressed. The term “regulatory sequence” includes promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence, as well as tissue-specific regulatory and/or inducible sequences. The design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of polypeptide desired, and the like. Expression vectors can be introduced into host cells to produce target polypeptides, including fusion polypeptides.

[0050] Recombinant expression vectors can be designed for expression of target polypeptides in prokaryotic or eukaryotic cells. For example, target polypeptides can be expressed in *E. coli*, insect cells (*e.g.*, using baculovirus expression vectors), yeast cells, or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology 185*, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

[0051] Expression of polypeptides in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion polypeptides. Fusion vectors add a number of amino acids to a polypeptide encoded therein, usually to the amino terminus of the recombinant polypeptide. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant polypeptide; 2) to increase the solubility of the recombinant polypeptide; and 3) to aid in the purification of the recombinant polypeptide by acting as a ligand in affinity purification. Often, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant polypeptide to enable separation of the recombinant polypeptide from the fusion moiety subsequent to purification of the fusion polypeptide. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith & Johnson, *Gene* 67: 31-40 (1988)), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding polypeptide, or polypeptide A, respectively, to the target recombinant polypeptide.

[0052] Purified fusion polypeptides can be used in screening assays and to generate antibodies specific for target polypeptides. In a therapeutic embodiment, fusion polypeptide expressed in a retroviral expression vector is used to infect bone marrow cells that are subsequently transplanted into irradiated recipients. The pathology of the subject recipient is then examined after sufficient time has passed (*e.g.*, six (6) weeks).

[0053] Expressing the polypeptide in host bacteria with an impaired capacity to proteolytically cleave the recombinant polypeptide is often used to maximize recombinant polypeptide expression (Gottesman, S., *Gene Expression Technology: Methods in Enzymology*, Academic Press, San Diego, California 185: 119-128 (1990)). Another strategy is to alter the nucleotide sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada *et al.*, *Nucleic Acids Res.* 20: 2111-2118 (1992)). Such alteration of nucleotide sequences can be carried out by standard DNA synthesis techniques.

[0054] When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. Recombinant mammalian expression vectors are often capable of directing expression of the nucleic acid in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Non-limiting examples of suitable tissue-specific promoters include an albumin promoter (liver-specific; Pinkert *et al.*, *Genes Dev.* 1: 268-277 (1987)), lymphoid-specific promoters (Calame & Eaton, *Adv. Immunol.* 43: 235-275 (1988)), promoters of T cell receptors (Winoto & Baltimore, *EMBO J.* 8: 729-733 (1989)) promoters of immunoglobulins

(Banerji *et al.*, *Cell* 33: 729-740 (1983); Queen & Baltimore, *Cell* 33: 741-748 (1983)), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne & Ruddle, *Proc. Natl. Acad. Sci. USA* 86: 5473-5477 (1989)), pancreas-specific promoters (Edlund *et al.*, *Science* 230: 912-916 (1985)), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are sometimes utilized, for example, the murine hox promoters (Kessel & Gruss, *Science* 249: 374-379 (1990)) and the α -fetopolypeptide promoter (Campes & Tilghman, *Genes Dev.* 3: 537-546 (1989)).

[0055] A *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleic acid also may be cloned into an expression vector in an antisense orientation. Regulatory sequences (*e.g.*, viral promoters and/or enhancers) operatively linked to a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleic acid cloned in the antisense orientation can be chosen for directing constitutive, tissue specific or cell type specific expression of antisense RNA in a variety of cell types. Antisense expression vectors can be in the form of a recombinant plasmid, phagemid or attenuated virus. For a discussion of the regulation of gene expression using antisense genes *see, e.g.*, Weintraub *et al.*, Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) (1986).

[0056] Also provided herein are host cells that include a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleotide sequence within a recombinant expression vector or a fragment of such a nucleotide sequence which facilitate homologous recombination into a specific site of the host cell genome. The terms "host cell" and "recombinant host cell" are used interchangeably herein. Such terms refer not only to the particular subject cell but rather also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein. A host cell can be any prokaryotic or eukaryotic cell. For example, a target polypeptide can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

[0057] Vectors can be introduced into host cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, transduction/infection, DEAE-dextran-mediated transfection, lipofection, or electroporation.

[0058] A host cell provided herein can be used to produce (*i.e.*, express) a target polypeptide or a substantially identical polypeptide thereof. Accordingly, further provided are methods for producing a

target polypeptide using host cells described herein. In one embodiment, the method includes culturing host cells into which a recombinant expression vector encoding a target polypeptide has been introduced in a suitable medium such that a target polypeptide is produced. In another embodiment, the method further includes isolating a target polypeptide from the medium or the host cell.

[0059] Also provided are cells or purified preparations of cells which include a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* transgene, or which otherwise misexpress target polypeptide. Cell preparations can consist of human or non-human cells, *e.g.*, rodent cells, *e.g.*, mouse or rat cells, rabbit cells, or pig cells. In preferred embodiments, the cell or cells include a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* transgene (*e.g.*, a heterologous form of a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* gene, such as a human gene expressed in non-human cells). The transgene can be misexpressed, *e.g.*, overexpressed or underexpressed. In other preferred embodiments, the cell or cells include a gene which misexpress an endogenous target polypeptide (*e.g.*, expression of a gene is disrupted, also known as a knockout). Such cells can serve as a model for studying disorders which are related to mutated or mis-expressed alleles or for use in drug screening. Also provided are human cells (*e.g.*, a hematopoietic stem cells) transfected with a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleic acid.

[0060] Also provided are cells or a purified preparation thereof (*e.g.*, human cells) in which an endogenous *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleic acid is under the control of a regulatory sequence that does not normally control the expression of the endogenous gene. The expression characteristics of an endogenous gene within a cell (*e.g.*, a cell line or microorganism) can be modified by inserting a heterologous DNA regulatory element into the genome of the cell such that the inserted regulatory element is operably linked to the corresponding endogenous gene. For example, an endogenous corresponding gene (*e.g.*, a gene which is “transcriptionally silent,” not normally expressed, or expressed only at very low levels) may be activated by inserting a regulatory element which is capable of promoting the expression of a normally expressed gene product in that cell. Techniques such as targeted homologous recombinations, can be used to insert the heterologous DNA as described in, *e.g.*, Chappel, US 5,272,071; WO 91/06667, published on May 16, 1991.

Transgenic Animals

[0061] Non-human transgenic animals that express a heterologous target polypeptide (*e.g.*, expressed from a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleic acid or substantially identical sequence thereof) can be generated. Such animals are useful for studying the function and/or activity of a target polypeptide and for identifying and/or evaluating modulators of the activity of *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleic

acids and encoded polypeptides. As used herein, a “transgenic animal” is a non-human animal such as a mammal (*e.g.*, a non-human primate such as chimpanzee, baboon, or macaque; an ungulate such as an equine, bovine, or caprine; or a rodent such as a rat, a mouse, or an Israeli sand rat), a bird (*e.g.*, a chicken or a turkey), an amphibian (*e.g.*, a frog, salamander, or newt), or an insect (*e.g.*, *Drosophila melanogaster*), in which one or more of the cells of the animal includes a transgene. A transgene is exogenous DNA or a rearrangement (*e.g.*, a deletion of endogenous chromosomal DNA) that is often integrated into or occurs in the genome of cells in a transgenic animal. A transgene can direct expression of an encoded gene product in one or more cell types or tissues of the transgenic animal, and other transgenes can reduce expression (*e.g.*, a knockout). Thus, a transgenic animal can be one in which an endogenous nucleic acid homologous to a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleic acid has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal (*e.g.*, an embryonic cell of the animal) prior to development of the animal.

[0062] Intronic sequences and polyadenylation signals can also be included in the transgene to increase expression efficiency of the transgene. One or more tissue-specific regulatory sequences can be operably linked to a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleotide sequence to direct expression of an encoded polypeptide to particular cells. A transgenic founder animal can be identified based upon the presence of a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleotide sequence in its genome and/or expression of encoded mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleotide sequence can further be bred to other transgenic animals carrying other transgenes.

[0063] Target polypeptides can be expressed in transgenic animals or plants by introducing, for example, a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleic acid into the genome of an animal that encodes the target polypeptide. In preferred embodiments the nucleic acid is placed under the control of a tissue specific promoter, *e.g.*, a milk or egg specific promoter, and recovered from the milk or eggs produced by the animal. Also included is a population of cells from a transgenic animal.

Target Polypeptides

[0064] Also featured herein are isolated target polypeptides, which are encoded by a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleotide sequence (*e.g.*, SEQ ID NO: 1), or a substantially identical nucleotide sequence thereof. Examples of *PADI2*, *APOB*, *IL1RL2*, *WASPIP*,

BVES, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* polypeptides are set forth in SEQ ID NO: 18-27. The term “polypeptide” as used herein includes proteins and peptides. An “isolated” or “purified” polypeptide or protein is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. In one embodiment, the language “substantially free” means preparation of a target polypeptide having less than about 30%, 20%, 10% and more preferably 5% (by dry weight), of non-target polypeptide (also referred to herein as a “contaminating protein”), or of chemical precursors or non-target chemicals. When the target polypeptide or a biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, specifically, where culture medium represents less than about 20%, sometimes less than about 10%, and often less than about 5% of the volume of the polypeptide preparation. Isolated or purified target polypeptide preparations are sometimes 0.01 milligrams or more or 0.1 milligrams or more, and often 1.0 milligrams or more and 10 milligrams or more in dry weight.

[0065] Further included herein are target polypeptide fragments. The polypeptide fragment may be a domain or part of a domain of a target polypeptide. The polypeptide fragment may have increased, decreased or unexpected biological activity. The polypeptide fragment is often 50 or fewer, 100 or fewer, or 200 or fewer amino acids in length, and is sometimes 300, 400, 500, 600, 700, or 900 or fewer amino acids in length. Shown in the table below are examples of polypeptide fragments, where approximate amino acid positions are shown in parenthesis (e.g., a Pellino domain starts at about amino acid 3 and ends at about amino acid 412).

RS_ID	Locus	SEQ ID NO.	Signal Peptide	Domain
910223	PADI2	18	none	
1367117	APOB	19	1-27	Apolipoprotein B48 mature peptide (1-2151) Lipoprotein amino terminal region (46-597) ATPase involved in DNA repair (2077-2583)
1024791	IL1RL2	20	1-19	Immunoglobulin C-2 Type (36-100; 137-197) TIR Domain (385-535) Neural cell adhesion molecule L1 (<53->295) Transmembrane Domain (336-358)
1465621	WASPIP	21	none	WASP-interacting protein VRP1/WIP

RS_ID	Locus	SEQ ID NO.	Signal Peptide	Domain
				(14->63)
1018810	BVES	22	none	Popeye protein conserved region (123-266)
242392	PELI2	23	none	Pellino (3-412)
8818	LOXL1	24	none	Lysyl oxidase (370-574)
1395486	CASPR4	25	none	Neurexin IV domain (3-1308) F5/8 type C domain (57-177) Laminin G domains (374-524; 475->750; 797-941; 1037-1176)
		26	none	Neurexin IV domain (1->721) F5/8 type C domain (29-149) Laminin G domains (169-314; 346-496; 579->662)
512294	GPR50	27	none	7 transmembrane receptor (rhodopsin family) (45..294) Microtubule-associated protein dynactin DCTN1/Glued (462..>587) Syndecan domain (485..>595)

[0066] Substantially identical target polypeptides may depart from the amino acid sequences of target polypeptides in different manners. For example, conservative amino acid modifications may be introduced at one or more positions in the amino acid sequences of target polypeptides. A “conservative amino acid substitution” is one in which the amino acid is replaced by another amino acid having a similar structure and/or chemical function. Families of amino acid residues having similar structures and functions are well known. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Also, essential and non-essential amino acids may be replaced. A “non-essential” amino acid is one that can be altered without abolishing or substantially altering the biological function of a target polypeptide, whereas altering an “essential” amino acid abolishes or substantially alters the biological function of a target polypeptide. Amino acids that are conserved among target polypeptides are typically essential amino acids. In certain embodiments, the polypeptide includes one or more non-

synonymous polymorphic variants associated with osteoarthritis, as described above (e.g., a threonine encoded by rs1367117 in an *APOB* polypeptide).

[0067] Also, target polypeptides may exist as chimeric or fusion polypeptides. As used herein, a target “chimeric polypeptide” or target “fusion polypeptide” includes a target polypeptide linked to a non-target polypeptide. A “non-target polypeptide” refers to a polypeptide having an amino acid sequence corresponding to a polypeptide which is not substantially identical to the target polypeptide, which includes, for example, a polypeptide that is different from the target polypeptide and derived from the same or a different organism. The target polypeptide in the fusion polypeptide can correspond to an entire or nearly entire target polypeptide or a fragment thereof. The non-target polypeptide can be fused to the N-terminus or C-terminus of the target polypeptide.

[0068] Fusion polypeptides can include a moiety having high affinity for a ligand. For example, the fusion polypeptide can be a GST-target fusion polypeptide in which the target sequences are fused to the C-terminus of the GST sequences, or a polyhistidine-target fusion polypeptide in which the target polypeptide is fused at the N- or C-terminus to a string of histidine residues. Such fusion polypeptides can facilitate purification of recombinant target polypeptide. Expression vectors are commercially available that already encode a fusion moiety (e.g., a GST polypeptide), and a nucleotide sequence in SEQ ID NO: 1-17, or a substantially identical nucleotide sequence thereof, can be cloned into an expression vector such that the fusion moiety is linked in-frame to the target polypeptide. Further, the fusion polypeptide can be a target polypeptide containing a heterologous signal sequence at its N-terminus. In certain host cells (e.g., mammalian host cells), expression, secretion, cellular internalization, and cellular localization of a target polypeptide can be increased through use of a heterologous signal sequence. Fusion polypeptides can also include all or a part of a serum polypeptide (e.g., an IgG constant region or human serum albumin).

[0069] Target polypeptides can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*. Administration of these target polypeptides can be used to affect the bioavailability of a substrate of the target polypeptide and may effectively increase target polypeptide biological activity in a cell. Target fusion polypeptides may be useful therapeutically for the treatment of disorders caused by, for example, (i) aberrant modification or mutation of a gene encoding a target polypeptide; (ii) mis-regulation of the gene encoding the target polypeptide; and (iii) aberrant post-translational modification of a target polypeptide. Also, target polypeptides can be used as immunogens to produce anti-target antibodies in a subject, to purify target polypeptide ligands or binding partners, and in screening assays to identify molecules which inhibit or enhance the interaction of a target polypeptide with a substrate.

[0070] In addition, polypeptides can be chemically synthesized using techniques known in the art (See, e.g., Creighton, 1983 *Proteins*. New York, N.Y.: W. H. Freeman and Company; and Hunkapiller et

al., (1984) Nature July 12 -18;310(5973):105-11). For example, a relative short fragment can be synthesized by use of a peptide synthesizer. Furthermore, if desired, non-classical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the fragment sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, α-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, ε-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β-alanine, fluoroamino acids, designer amino acids such as β-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

[0071] Polypeptides and polypeptide fragments sometimes are differentially modified during or after translation, *e.g.*, by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH₄; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; and the like. Additional post-translational modifications include, for example, N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of prokaryotic host cell expression. The polypeptide fragments may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the polypeptide.

[0072] Also provided are chemically modified derivatives of polypeptides that can provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (*see e.g.*, U.S. Pat. No: 4,179,337. The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

[0073] The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term “about” indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (*e.g.*, the duration of sustained release desired, the effects, if

any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog).

[0074] The polymers should be attached to the polypeptide with consideration of effects on functional or antigenic domains of the polypeptide. There are a number of attachment methods available to those skilled in the art (*e.g.*, EP 0 401 384 (coupling PEG to G-CSF) and Malik et al. (1992) Exp Hematol. September;20(8):1028-35 (pegylation of GM-CSF using tresyl chloride)). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues, glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. For therapeutic purposes, the attachment sometimes is at an amino group, such as attachment at the N-terminus or lysine group.

[0075] Proteins can be chemically modified at the N-terminus. Using polyethylene glycol as an illustration of such a composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, and the like), the proportion of polyethylene glycol molecules to protein (polypeptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (*i.e.*, separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus may be accomplished by reductive alkylation, which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

Substantially Identical Nucleic Acids and Polypeptides

[0076] Nucleotide sequences and polypeptide sequences that are substantially identical to a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleotide sequence and the target polypeptide sequences encoded by those nucleotide sequences, respectively, are included herein. The term “substantially identical” as used herein refers to two or more nucleic acids or polypeptides sharing one or more identical nucleotide sequences or polypeptide sequences, respectively. Included are nucleotide sequences or polypeptide sequences that are 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 95% or more (each often within a 1%,

2%, 3% or 4% variability) identical to a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleotide sequence or the encoded target polypeptide amino acid sequences. One test for determining whether two nucleic acids are substantially identical is to determine the percent of identical nucleotide sequences or polypeptide sequences shared between the nucleic acids or polypeptides.

[0077] Calculations of sequence identity are often performed as follows. Sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). The length of a reference sequence aligned for comparison purposes is sometimes 30% or more, 40% or more, 50% or more, often 60% or more, and more often 70% or more, 80% or more, 90% or more, or 100% of the length of the reference sequence. The nucleotides or amino acids at corresponding nucleotide or polypeptide positions, respectively, are then compared among the two sequences. When a position in the first sequence is occupied by the same nucleotide or amino acid as the corresponding position in the second sequence, the nucleotides or amino acids are deemed to be identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, introduced for optimal alignment of the two sequences.

[0078] Comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. Percent identity between two amino acid or nucleotide sequences can be determined using the algorithm of Meyers & Miller, *CABIOS* 4: 11-17 (1989), which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. Also, percent identity between two amino acid sequences can be determined using the Needleman & Wunsch, *J. Mol. Biol.* 48: 444-453 (1970) algorithm which has been incorporated into the GAP program in the GCG software package (available at the http address www.gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. Percent identity between two nucleotide sequences can be determined using the GAP program in the GCG software package (available at http address www.gcg.com), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A set of parameters often used is a Blossum 62 scoring matrix with a gap open penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

[0079] Another manner for determining if two nucleic acids are substantially identical is to assess whether a polynucleotide homologous to one nucleic acid will hybridize to the other nucleic acid under stringent conditions. As use herein, the term "stringent conditions" refers to conditions for hybridization and washing. Stringent conditions are known to those skilled in the art and can be found in *Current*

Protocols in Molecular Biology, John Wiley & Sons, N.Y. , 6.3.1-6.3.6 (1989). Aqueous and non-aqueous methods are described in that reference and either can be used. An example of stringent hybridization conditions is hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 50°C. Another example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 55°C. A further example of stringent hybridization conditions is hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 60°C. Often, stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 65°C. More often, stringency conditions are 0.5M sodium phosphate, 7% SDS at 65°C, followed by one or more washes at 0.2X SSC, 1% SDS at 65°C.

[0080] An example of a substantially identical nucleotide sequence to a nucleotide sequence in SEQ ID NO: 1-17 is one that has a different nucleotide sequence but still encodes the same polypeptide sequence encoded by the nucleotide sequence in SEQ ID NO: 1-17. Another example is a nucleotide sequence that encodes a polypeptide having a polypeptide sequence that is more than 70% or more identical to, sometimes more than 75% or more, 80% or more, or 85% or more identical to, and often more than 90% or more and 95% or more identical to a polypeptide sequence encoded by a nucleotide sequence in SEQ ID NO: 1-17.

[0081] Nucleotide sequences in SEQ ID NO: 1-17 and amino acid sequences of encoded polypeptides can be used as “query sequences” to perform a search against public databases to identify other family members or related sequences, for example. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul *et al.*, *J. Mol. Biol.* 215: 403-10 (1990). BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to nucleotide sequences in SEQ ID NO: 1-17. BLAST polypeptide searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to polypeptides encoded by the nucleotide sequences of SEQ ID NO: 1-17. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, *Nucleic Acids Res.* 25(17): 3389-3402 (1997). When utilizing BLAST and Gapped BLAST programs, default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used (*see* the http address www.ncbi.nlm.nih.gov).

[0082] A nucleic acid that is substantially identical to a nucleotide sequence in SEQ ID NO: 1-17 may include polymorphic sites at positions equivalent to those described herein when the sequences are aligned. For example, using the alignment procedures described herein, SNPs in a sequence substantially

identical to a sequence in SEQ ID NO: 1-17 can be identified at nucleotide positions that match (*i.e.*, align) with nucleotides at SNP positions in each nucleotide sequence in SEQ ID NO: 1-17. Also, where a polymorphic variation results in an insertion or deletion, insertion or deletion of a nucleotide sequence from a reference sequence can change the relative positions of other polymorphic sites in the nucleotide sequence.

[0083] Substantially identical nucleotide and polypeptide sequences include those that are naturally occurring, such as allelic variants (same locus), splice variants, homologs (different locus), and orthologs (different organism) or can be non-naturally occurring. Non-naturally occurring variants can be generated by mutagenesis techniques, including those applied to polynucleotides, cells, or organisms. The variants can contain nucleotide substitutions, deletions, inversions and insertions. Variation can occur in either or both the coding and non-coding regions. The variations can produce both conservative and non-conservative amino acid substitutions (as compared in the encoded product). Orthologs, homologs, allelic variants, and splice variants can be identified using methods known in the art. These variants normally comprise a nucleotide sequence encoding a polypeptide that is 50% or more, about 55% or more, often about 70-75% or more or about 80-85% or more, and sometimes about 90-95% or more identical to the amino acid sequences of target polypeptides or a fragment thereof. Such nucleic acid molecules can readily be identified as being able to hybridize under stringent conditions to a nucleotide sequence in SEQ ID NO: 1-17 or a fragment of this sequence. Nucleic acid molecules corresponding to orthologs, homologs, and allelic variants of a nucleotide sequence in SEQ ID NO: 1-17 can further be identified by mapping the sequence to the same chromosome or locus as the nucleotide sequence in SEQ ID NO: 1-17.

[0084] Also, substantially identical nucleotide sequences may include codons that are altered with respect to the naturally occurring sequence for enhancing expression of a target polypeptide in a particular expression system. For example, the nucleic acid can be one in which one or more codons are altered, and often 10% or more or 20% or more of the codons are altered for optimized expression in bacteria (*e.g.*, *E. coli.*), yeast (*e.g.*, *S. cerevisiae*), human (*e.g.*, 293 cells), insect, or rodent (*e.g.*, hamster) cells.

Methods for Identifying Risk of osteoarthritis

[0085] Methods for prognosing and diagnosing osteoarthritis are included herein. These methods include detecting the presence or absence of one or more polymorphic variations in a nucleotide sequence associated with osteoarthritis, such as variants in or around the loci set forth herein, or a substantially identical sequence thereof, in a sample from a subject, where the presence of a polymorphic variant described herein is indicative of a risk of osteoarthritis. Determining a risk of osteoarthritis sometimes

refers to determining whether an individual is at an increased risk of osteoarthritis (e.g., intermediate risk or higher risk).

[0086] Thus, featured herein is a method for identifying a subject who is at risk of osteoarthritis, which comprises detecting an aberration associated with osteoarthritis in a nucleic acid sample from the subject. An embodiment is a method for detecting a risk of osteoarthritis in a subject, which comprises detecting the presence or absence of a polymorphic variation associated with osteoarthritis at a polymorphic site in a nucleotide sequence in a nucleic acid sample from a subject, where the nucleotide sequence comprises a polynucleotide sequence selected from the group consisting of: (a) a nucleotide sequence of SEQ ID NO: 1-17; (b) a nucleotide sequence which encodes a polypeptide consisting of an amino acid sequence encoded by a nucleotide sequence of SEQ ID NO: 1-17; (c) a nucleotide sequence which encodes a polypeptide that is 90% or more identical to an amino acid sequence encoded by a nucleotide sequence of SEQ ID NO: 1-17, or a nucleotide sequence about 90% or more identical to a nucleotide sequence of SEQ ID NO: 1-17; and (d) a fragment of a nucleotide sequence of (a), (b), or (c) comprising the polymorphic site; whereby the presence of the polymorphic variation is indicative of a predisposition to osteoarthritis in the subject. In certain embodiments, polymorphic variants at the positions described herein are detected for determining a risk of osteoarthritis, and polymorphic variants at positions in linkage disequilibrium with these positions are detected for determining a risk of osteoarthritis. As used herein, "SEQ ID NO: 1-17" refers to individual sequences in SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 or 17, each sequence being separately applicable to embodiments described herein.

[0087] Risk of osteoarthritis sometimes is expressed as a probability, such as an odds ratio, percentage, or risk factor. Risk often is based upon the presence or absence of one or more polymorphic variants described herein, and also may be based in part upon phenotypic traits of the individual being tested. Methods for calculating risk based upon patient data are well known (*see, e.g.,* Agresti, *Categorical Data Analysis*, 2nd Ed. 2002. Wiley). Allelotyping and genotyping analyses may be carried out in populations other than those exemplified herein to enhance the predictive power of the prognostic method. These further analyses are executed in view of the exemplified procedures described herein, and may be based upon the same polymorphic variations or additional polymorphic variations.

[0088] In certain embodiments, determining the presence of a combination of two or more polymorphic variants associated with osteoarthritis in one or more genetic loci (e.g., one or more genes) of the sample is determined to identify, quantify and/or estimate, risk of osteoarthritis. The risk often is the probability of having or developing osteoarthritis. The risk sometimes is expressed as a relative risk with respect to a population average risk of osteoarthritis, and sometimes is expressed as a relative risk with respect to the lowest risk group. Such relative risk assessments often are based upon penetrance

values determined by statistical methods, and are particularly useful to clinicians and insurance companies for assessing risk of osteoarthritis (e.g., a clinician can target appropriate detection, prevention and therapeutic regimens to a patient after determining the patient's risk of osteoarthritis, and an insurance company can fine tune actuarial tables based upon population genotype assessments of osteoarthritis risk). Risk of osteoarthritis sometimes is expressed as an odds ratio, which is the odds of a particular person having a genotype has or will develop osteoarthritis with respect to another genotype group (e.g., the most disease protective genotype or population average). In related embodiments, the determination is utilized to identify a subject at risk of osteoarthritis. In an embodiment, two or more polymorphic variations are detected in two or more regions in human genomic DNA associated with increased risk of osteoarthritis, such as a locus containing a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* region, for example. In certain embodiments, 3 or more, or 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 or more polymorphic variants are detected in the sample. In specific embodiments, polymorphic variants are detected in a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* region, for example. In another embodiment, polymorphic variants are detected at two or three positions selected from the group consisting of position 52511 in SEQ ID NO: 2 and positions 30900, 76512 and/or 94155 in SEQ ID NO: 3. In certain embodiments, polymorphic variants are detected at other genetic loci (e.g., the polymorphic variants can be detected in *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* in addition to other loci or only in other loci), where the other loci include but are not limited to those described in concurrently-filed patent applications having attorney docket number 524593008700, 524593008900, 524593009000, 524593009100 or 524593009200, each of which is incorporated herein by reference in its entirety.

[0089] Results from prognostic tests may be combined with other test results to diagnose osteoarthritis. For example, prognostic results may be gathered, a patient sample may be ordered based on a determined predisposition to osteoarthritis, the patient sample is analyzed, and the results of the analysis may be utilized to diagnose osteoarthritis. Also osteoarthritis diagnostic method can be developed from studies used to generate prognostic methods in which populations are stratified into subpopulations having different progressions of osteoarthritis. In another embodiment, prognostic results may be gathered, a patient's risk factors for developing osteoarthritis (e.g., age, weight, race, diet) analyzed, and a patient sample may be ordered based on a determined predisposition to osteoarthritis.

[0090] The nucleic acid sample typically is isolated from a biological sample obtained from a subject. For example, nucleic acid can be isolated from blood, saliva, sputum, urine, cell scrapings, and biopsy tissue. The nucleic acid sample can be isolated from a biological sample using standard techniques, such as the technique described in Example 2. As used herein, the term "subject" refers

primarily to humans but also refers to other mammals such as dogs, cats, and ungulates (*e.g.*, cattle, sheep, and swine). Subjects also include avians (*e.g.*, chickens and turkeys), reptiles, and fish (*e.g.*, salmon), as embodiments described herein can be adapted to nucleic acid samples isolated from any of these organisms. The nucleic acid sample may be isolated from the subject and then directly utilized in a method for determining the presence of a polymorphic variant, or alternatively, the sample may be isolated and then stored (*e.g.*, frozen) for a period of time before being subjected to analysis.

[0091] The presence or absence of a polymorphic variant is determined using one or both chromosomal complements represented in the nucleic acid sample. Determining the presence or absence of a polymorphic variant in both chromosomal complements represented in a nucleic acid sample from a subject having a copy of each chromosome is useful for determining the zygosity of an individual for the polymorphic variant (*i.e.*, whether the individual is homozygous or heterozygous for the polymorphic variant). Any oligonucleotide-based diagnostic may be utilized to determine whether a sample includes the presence or absence of a polymorphic variant in a sample. For example, primer extension methods, ligase sequence determination methods (*e.g.*, U.S. Pat. Nos. 5,679,524 and 5,952,174, and WO 01/27326), mismatch sequence determination methods (*e.g.*, U.S. Pat. Nos. 5,851,770; 5,958,692; 6,110,684; and 6,183,958), microarray sequence determination methods, restriction fragment length polymorphism (RFLP), single strand conformation polymorphism detection (SSCP) (*e.g.*, U.S. Pat. Nos. 5,891,625 and 6,013,499), PCR-based assays (*e.g.*, TAQMAN[®] PCR System (Applied Biosystems)), and nucleotide sequencing methods may be used.

[0092] Oligonucleotide extension methods typically involve providing a pair of oligonucleotide primers in a polymerase chain reaction (PCR) or in other nucleic acid amplification methods for the purpose of amplifying a region from the nucleic acid sample that comprises the polymorphic variation. One oligonucleotide primer is complementary to a region 3' of the polymorphism and the other is complementary to a region 5' of the polymorphism. A PCR primer pair may be used in methods disclosed in U.S. Pat. Nos. 4,683,195; 4,683,202, 4,965,188; 5,656,493; 5,998,143; 6,140,054; WO 01/27327; and WO 01/27329 for example. PCR primer pairs may also be used in any commercially available machines that perform PCR, such as any of the GENEAMP[®] Systems available from Applied Biosystems. Also, those of ordinary skill in the art will be able to design oligonucleotide primers based upon a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXLI*, *CASPR4* or *GPR50* nucleotide sequence using knowledge available in the art.

[0093] Also provided is an extension oligonucleotide that hybridizes to the amplified fragment adjacent to the polymorphic variation. As used herein, the term "adjacent" refers to the 3' end of the extension oligonucleotide being often 1 nucleotide from the 5' end of the polymorphic site, and sometimes 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides from the 5' end of the polymorphic site, in the nucleic

acid when the extension oligonucleotide is hybridized to the nucleic acid. The extension oligonucleotide then is extended by one or more nucleotides, and the number and/or type of nucleotides that are added to the extension oligonucleotide determine whether the polymorphic variant is present. Oligonucleotide extension methods are disclosed, for example, in U.S. Pat. Nos. 4,656,127; 4,851,331; 5,679,524; 5,834,189; 5,876,934; 5,908,755; 5,912,118; 5,976,802; 5,981,186; 6,004,744; 6,013,431; 6,017,702; 6,046,005; 6,087,095; 6,210,891; and WO 01/20039. Oligonucleotide extension methods using mass spectrometry are described, for example, in U.S. Pat. Nos. 5,547,835; 5,605,798; 5,691,141; 5,849,542; 5,869,242; 5,928,906; 6,043,031; and 6,194,144, and a method often utilized is described herein in Example 2.

[0094] A microarray can be utilized for determining whether a polymorphic variant is present or absent in a nucleic acid sample. A microarray may include any oligonucleotides described herein, and methods for making and using oligonucleotide microarrays suitable for diagnostic use are disclosed in U.S. Pat. Nos. 5,492,806; 5,525,464; 5,589,330; 5,695,940; 5,849,483; 6,018,041; 6,045,996; 6,136,541; 6,142,681; 6,156,501; 6,197,506; 6,223,127; 6,225,625; 6,229,911; 6,239,273; WO 00/52625; WO 01/25485; and WO 01/29259. The microarray typically comprises a solid support and the oligonucleotides may be linked to this solid support by covalent bonds or by non-covalent interactions. The oligonucleotides may also be linked to the solid support directly or by a spacer molecule. A microarray may comprise one or more oligonucleotides complementary to a polymorphic site set forth herein.

[0095] A kit also may be utilized for determining whether a polymorphic variant is present or absent in a nucleic acid sample. A kit often comprises one or more pairs of oligonucleotide primers useful for amplifying a fragment of a nucleotide sequence of SEQ ID NO: 1-17 or a substantially identical sequence thereof, where the fragment includes a polymorphic site. The kit sometimes comprises a polymerizing agent, for example, a thermostable nucleic acid polymerase such as one disclosed in U.S. Pat. Nos. 4,889,818 or 6,077,664. Also, the kit often comprises an elongation oligonucleotide that hybridizes to a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXLI*, *CASPR4* or *GPR50* nucleotide sequence in a nucleic acid sample adjacent to the polymorphic site. Where the kit includes an elongation oligonucleotide, it also often comprises chain elongating nucleotides, such as dATP, dTTP, dGTP, dCTP, and dITP, including analogs of dATP, dTTP, dGTP, dCTP and dITP, provided that such analogs are substrates for a thermostable nucleic acid polymerase and can be incorporated into a nucleic acid chain elongated from the extension oligonucleotide. Along with chain elongating nucleotides would be one or more chain terminating nucleotides such as ddATP, ddTTP, ddGTP, ddCTP, and the like. In an embodiment, the kit comprises one or more oligonucleotide primer pairs, a polymerizing agent, chain

elongating nucleotides, at least one elongation oligonucleotide, and one or more chain terminating nucleotides. Kits optionally include buffers, vials, microtiter plates, and instructions for use.

[0096] An individual identified as being at risk of osteoarthritis may be heterozygous or homozygous with respect to the allele associated with a higher risk of osteoarthritis. A subject homozygous for an allele associated with an increased risk of osteoarthritis is at a comparatively high risk of osteoarthritis, a subject heterozygous for an allele associated with an increased risk of osteoarthritis is at a comparatively intermediate risk of osteoarthritis, and a subject homozygous for an allele associated with a decreased risk of osteoarthritis is at a comparatively low risk of osteoarthritis. A genotype may be assessed for a complementary strand, such that the complementary nucleotide at a particular position is detected.

[0097] Also featured are methods for determining risk of osteoarthritis and/or identifying a subject at risk of osteoarthritis by contacting a polypeptide or protein encoded by a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleotide sequence from a subject with an antibody that specifically binds to an epitope associated with increased risk of osteoarthritis in the polypeptide.

Applications of Prognostic and Diagnostic Results to Pharmacogenomic Methods

[0098] Pharmacogenomics is a discipline that involves tailoring a treatment for a subject according to the subject's genotype as a particular treatment regimen may exert a differential effect depending upon the subject's genotype. For example, based upon the outcome of a prognostic test described herein, a clinician or physician may target pertinent information and preventative or therapeutic treatments to a subject who would be benefited by the information or treatment and avoid directing such information and treatments to a subject who would not be benefited (*e.g.*, the treatment has no therapeutic effect and/or the subject experiences adverse side effects).

[0099] The following is an example of a pharmacogenomic embodiment. A particular treatment regimen can exert a differential effect depending upon the subject's genotype. Where a candidate therapeutic exhibits a significant interaction with a major allele and a comparatively weak interaction with a minor allele (*e.g.*, an order of magnitude or greater difference in the interaction), such a therapeutic typically would not be administered to a subject genotyped as being homozygous for the minor allele, and sometimes not administered to a subject genotyped as being heterozygous for the minor allele. In another example, where a candidate therapeutic is not significantly toxic when administered to subjects who are homozygous for a major allele but is comparatively toxic when administered to subjects heterozygous or homozygous for a minor allele, the candidate therapeutic is not typically administered to subjects who are genotyped as being heterozygous or homozygous with respect to the minor allele.

[0100] The methods described herein are applicable to pharmacogenomic methods for preventing, alleviating or treating osteoarthritis. For example, a nucleic acid sample from an individual may be subjected to a prognostic test described herein. Where one or more polymorphic variations associated with increased risk of osteoarthritis are identified in a subject, information for preventing or treating osteoarthritis and/or one or more osteoarthritis treatment regimens then may be prescribed to that subject.

[0101] In certain embodiments, a treatment or preventative regimen is specifically prescribed and/or administered to individuals who will most benefit from it based upon their risk of developing osteoarthritis assessed by the methods described herein. Thus, provided are methods for identifying a subject predisposed to osteoarthritis and then prescribing a therapeutic or preventative regimen to individuals identified as having a predisposition. Thus, certain embodiments are directed to a method for reducing osteoarthritis in a subject, which comprises: detecting the presence or absence of a polymorphic variant associated with osteoarthritis in a nucleotide sequence in a nucleic acid sample from a subject, where the nucleotide sequence comprises a polynucleotide sequence selected from the group consisting of: (a) a nucleotide sequence of SEQ ID NO: 1-17; (b) a nucleotide sequence which encodes a polypeptide consisting of an amino acid sequence encoded by a nucleotide sequence of SEQ ID NO: 1-17; (c) a nucleotide sequence which encodes a polypeptide that is 90% or more identical to an amino acid sequence encoded by a nucleotide sequence of SEQ ID NO: 1-17, or a nucleotide sequence about 90% or more identical to a nucleotide sequence of SEQ ID NO: 1-17; and (d) a fragment of a polynucleotide sequence of (a), (b), or (c); and prescribing or administering a treatment regimen to a subject from whom the sample originated where the presence of a polymorphic variation associated with osteoarthritis is detected in the nucleotide sequence. In these methods, predisposition results may be utilized in combination with other test results to diagnose osteoarthritis.

[0102] Certain preventative treatments often are prescribed to subjects having a predisposition to osteoarthritis and where the subject is diagnosed with osteoarthritis or is diagnosed as having symptoms indicative of an early stage of osteoarthritis. The treatment sometimes is preventative (e.g., is prescribed or administered to reduce the probability that osteoarthritis arises or progresses), sometimes is therapeutic, and sometimes delays, alleviates or halts the progression of osteoarthritis. Any known preventative or therapeutic treatment for alleviating or preventing the occurrence of osteoarthritis is prescribed and/or administered. For example, the treatment often is directed to decreasing pain and improving joint movement. Examples of OA treatments include exercises to keep joints flexible and improve muscle strength. Different medications to control pain, including corticosteroids and nonsteroidal anti-inflammatory drugs (NSAIDs, e.g., Voltaren); cyclooxygenase-2 (COX-2) inhibitors (e.g., Celebrex, Vioxx, Mobic, and Bextra); monoclonal antibodies (e.g., Remicade); tumor necrosis factor inhibitors (e.g., Enbrel); or injections of glucocorticoids, hyaluronic acid or chondroitin sulfate into

joints that are inflamed and not responsive to NSAIDs. Orally administered chondroitin sulfate also may be used as a therapeutic, as it may increase hyaluronic acid levels and viscosity of synovial fluid, and decrease collagenase levels in synovial fluid. Also, glucosamine can serve as an OA therapeutic as delivering it into joints may inhibit enzymes involved in cartilage degradation and enhance the production of hyaluronic acid. For mild pain without inflammation, acetaminophen may be used. Other treatments include: heat/cold therapy for temporary pain relief; joint protection to prevent strain or stress on painful joints; surgery to relieve chronic pain in damaged joints; and weight control to prevent extra stress on weight-bearing joints.

[0103] As therapeutic approaches for treating osteoarthritis continue to evolve and improve, the goal of treatments for osteoarthritis related disorders is to intervene even before clinical signs first manifest. Thus, genetic markers associated with susceptibility to osteoarthritis prove useful for early diagnosis, prevention and treatment of osteoarthritis.

[0104] As osteoarthritis preventative and treatment information can be specifically targeted to subjects in need thereof (*e.g.*, those at risk of developing osteoarthritis or those in an early stage of osteoarthritis), provided herein is a method for preventing or reducing the risk of developing osteoarthritis in a subject, which comprises: (a) detecting the presence or absence of a polymorphic variation associated with osteoarthritis at a polymorphic site in a nucleotide sequence in a nucleic acid sample from a subject; (b) identifying a subject with a predisposition to osteoarthritis, whereby the presence of the polymorphic variation is indicative of a predisposition to osteoarthritis in the subject; and (c) if such a predisposition is identified, providing the subject with information about methods or products to prevent or reduce osteoarthritis or to delay the onset of osteoarthritis. Also provided is a method of targeting information or advertising to a subpopulation of a human population based on the subpopulation being genetically predisposed to a disease or condition, which comprises: (a) detecting the presence or absence of a polymorphic variation associated with osteoarthritis at a polymorphic site in a nucleotide sequence in a nucleic acid sample from a subject; (b) identifying the subpopulation of subjects in which the polymorphic variation is associated with osteoarthritis; and (c) providing information only to the subpopulation of subjects about a particular product which may be obtained and consumed or applied by the subject to help prevent or delay onset of the disease or condition.

[0105] Pharmacogenomics methods also may be used to analyze and predict a response to osteoarthritis treatment or a drug. For example, if pharmacogenomics analysis indicates a likelihood that an individual will respond positively to osteoarthritis treatment with a particular drug, the drug may be administered to the individual. Conversely, if the analysis indicates that an individual is likely to respond negatively to treatment with a particular drug, an alternative course of treatment may be prescribed. A negative response may be defined as either the absence of an efficacious response or the presence of toxic

side effects. The response to a therapeutic treatment can be predicted in a background study in which subjects in any of the following populations are genotyped: a population that responds favorably to a treatment regimen, a population that does not respond significantly to a treatment regimen, and a population that responds adversely to a treatment regimen (*e.g.*, exhibits one or more side effects). These populations are provided as examples and other populations and subpopulations may be analyzed. Based upon the results of these analyses, a subject is genotyped to predict whether he or she will respond favorably to a treatment regimen, not respond significantly to a treatment regimen, or respond adversely to a treatment regimen.

[0106] The tests described herein also are applicable to clinical drug trials. One or more polymorphic variants indicative of response to an agent for treating osteoarthritis or to side effects to an agent for treating osteoarthritis may be identified using the methods described herein. Thereafter, potential participants in clinical trials of such an agent may be screened to identify those individuals most likely to respond favorably to the drug and exclude those likely to experience side effects. In that way, the effectiveness of drug treatment may be measured in individuals who respond positively to the drug, without lowering the measurement as a result of the inclusion of individuals who are unlikely to respond positively in the study and without risking undesirable safety problems.

[0107] Thus, another embodiment is a method of selecting an individual for inclusion in a clinical trial of a treatment or drug comprising the steps of: (a) obtaining a nucleic acid sample from an individual; (b) determining the identity of a polymorphic variation which is associated with a positive response to the treatment or the drug, or at least one polymorphic variation which is associated with a negative response to the treatment or the drug in the nucleic acid sample, and (c) including the individual in the clinical trial if the nucleic acid sample contains said polymorphic variation associated with a positive response to the treatment or the drug or if the nucleic acid sample lacks said polymorphic variation associated with a negative response to the treatment or the drug. In addition, the methods described herein for selecting an individual for inclusion in a clinical trial of a treatment or drug encompass methods with any further limitation described in this disclosure, or those following, specified alone or in any combination. The polymorphic variation may be in a sequence selected individually or in any combination from the group consisting of (i) a nucleotide sequence of SEQ ID NO: 1-17; (ii) a nucleotide sequence which encodes a polypeptide consisting of an amino acid sequence encoded by a nucleotide sequence of SEQ ID NO: 1-17; (iii) a nucleotide sequence which encodes a polypeptide that is 90% or more identical to an amino acid sequence encoded by a nucleotide sequence of SEQ ID NO: 1-17, or a nucleotide sequence about 90% or more identical to a nucleotide sequence of SEQ ID NO: 1-17; and (iv) a fragment of a polynucleotide sequence of (i), (ii), or (iii) comprising the polymorphic site. The including step (c) optionally comprises administering the drug or the treatment to the individual if the

nucleic acid sample contains the polymorphic variation associated with a positive response to the treatment or the drug and the nucleic acid sample lacks said biallelic marker associated with a negative response to the treatment or the drug.

[0108] Also provided herein is a method of partnering between a diagnostic/prognostic testing provider and a provider of a consumable product, which comprises: (a) the diagnostic/prognostic testing provider detects the presence or absence of a polymorphic variation associated with osteoarthritis at a polymorphic site in a nucleotide sequence in a nucleic acid sample from a subject; (b) the diagnostic/prognostic testing provider identifies the subpopulation of subjects in which the polymorphic variation is associated with osteoarthritis; (c) the diagnostic/prognostic testing provider forwards information to the subpopulation of subjects about a particular product which may be obtained and consumed or applied by the subject to help prevent or delay onset of the disease or condition; and (d) the provider of a consumable product forwards to the diagnostic test provider a fee every time the diagnostic/prognostic test provider forwards information to the subject as set forth in step (c) above.

Compositions Comprising Osteoarthritis-Directed Molecules

[0109] Featured herein is a composition comprising a cell from a subject having osteoarthritis or at risk of osteoarthritis and one or more molecules specifically directed and targeted to a nucleic acid comprising a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleotide sequence or amino acid sequence. Such directed molecules include, but are not limited to, a compound that binds to a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleotide sequence or amino acid sequence referenced herein; a RNAi or siRNA molecule having a strand complementary or substantially complementary to a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleotide sequence (e.g., hybridizes to a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleotide sequence under conditions of high stringency); an antisense nucleic acid complementary or substantially complementary to an RNA encoded by a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleotide sequence (e.g., hybridizes to a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleotide sequence under conditions of high stringency); a ribozyme that hybridizes to a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleotide sequence (e.g., hybridizes to a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleotide sequence under conditions of high stringency); a nucleic acid aptamer that specifically binds a polypeptide encoded by *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleotide sequence; and an antibody that specifically binds to a polypeptide encoded by *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleotide sequence or binds to a nucleic

acid having such a nucleotide sequence. In an embodiment, the antibody selectively binds to an epitope comprising an amino acid encoded by rs1367117 in *APOB*. In specific embodiments, the osteoarthritis directed molecule interacts with a nucleic acid or polypeptide variant associated with osteoarthritis, such as variants referenced herein. In other embodiments, the osteoarthritis directed molecule interacts with a polypeptide involved in a signal pathway of a polypeptide encoded by a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleotide sequence, or a nucleic acid comprising such a nucleotide sequence.

[0110] Compositions sometimes include an adjuvant known to stimulate an immune response, and in certain embodiments, an adjuvant that stimulates a T-cell lymphocyte response. Adjuvants are known, including but not limited to an aluminum adjuvant (e.g., aluminum hydroxide); a cytokine adjuvant or adjuvant that stimulates a cytokine response (e.g., interleukin (IL)-12 and/or gamma-interferon cytokines); a Freund-type mineral oil adjuvant emulsion (e.g., Freund's complete or incomplete adjuvant); a synthetic lipid compound; a copolymer adjuvant (e.g., TitreMax); a saponin; Quil A; a liposome; an oil-in-water emulsion (e.g., an emulsion stabilized by Tween 80 and pluronic polyoxyethylene/polyoxypropylene block copolymer (Syntex Adjuvant Formulation); TitreMax; detoxified endotoxin (MPL) and mycobacterial cell wall components (TDW, CWS) in 2% squalene (Ribi Adjuvant System)); a muramyl dipeptide; an immune-stimulating complex (ISCOM, e.g., an Ag-modified saponin/cholesterol micelle that forms stable cage-like structure); an aqueous phase adjuvant that does not have a depot effect (e.g., Gerbu adjuvant); a carbohydrate polymer (e.g., AdjuPrime); L-tyrosine; a manide-oleate compound (e.g., Montanide); an ethylene-vinyl acetate copolymer (e.g., Elvax 40W1,2); or lipid A, for example. Such compositions are useful for generating an immune response against osteoarthritis directed molecule (e.g., an HLA-binding subsequence within a polypeptide encoded by a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleotide sequence). In such methods, a peptide having an amino acid subsequence of a polypeptide encoded by a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleotide sequence is delivered to a subject, where the subsequence binds to an HLA molecule and induces a CTL lymphocyte response. The peptide sometimes is delivered to the subject as an isolated peptide or as a minigene in a plasmid that encodes the peptide. Methods for identifying HLA-binding subsequences in such polypeptides are known (see e.g., publication WO02/20616 and PCT application US98/01373 for methods of identifying such sequences).

[0111] The cell may be in a group of cells cultured *in vitro* or in a tissue maintained *in vitro* or present in an animal *in vivo* (e.g., a rat, mouse, ape or human). In certain embodiments, a composition comprises a component from a cell such as a nucleic acid molecule (e.g., genomic DNA), a protein mixture or isolated protein, for example. The aforementioned compositions have utility in diagnostic,

prognostic and pharmacogenomic methods described previously and in therapeutics described hereafter. Certain osteoarthritis directed molecules are described in greater detail below.

Compounds

[0112] Compounds can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; peptoid libraries (libraries of molecules having the functionalities of peptides, but with a novel, non-peptide backbone which are resistant to enzymatic degradation but which nevertheless remain bioactive (see, e.g., Zuckermann et al., *J. Med. Chem.* 37: 2678-85 (1994)); spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; “one-bead one-compound” library methods; and synthetic library methods using affinity chromatography selection. Biological library and peptoid library approaches are typically limited to peptide libraries, while the other approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, *Anticancer Drug Des.* 12: 145, (1997)). Examples of methods for synthesizing molecular libraries are described, for example, in DeWitt et al., *Proc. Natl. Acad. Sci. U.S.A.* 90: 6909 (1993); Erb et al., *Proc. Natl. Acad. Sci. USA* 91: 11422 (1994); Zuckermann et al., *J. Med. Chem.* 37: 2678 (1994); Cho et al., *Science* 261: 1303 (1993); Carrell et al., *Angew. Chem. Int. Ed. Engl.* 33: 2059 (1994); Carell et al., *Angew. Chem. Int. Ed. Engl.* 33: 2061 (1994); and in Gallop et al., *J. Med. Chem.* 37: 1233 (1994).

[0113] Libraries of compounds may be presented in solution (e.g., Houghten, *Biotechniques* 13: 412-421 (1992)), or on beads (Lam, *Nature* 354: 82-84 (1991)), chips (Fodor, *Nature* 364: 555-556 (1993)), bacteria or spores (Ladner, United States Patent No. 5,223,409), plasmids (Cull et al., *Proc. Natl. Acad. Sci. USA* 89: 1865-1869 (1992)) or on phage (Scott and Smith, *Science* 249: 386-390 (1990); Devlin, *Science* 249: 404-406 (1990); Cwirla et al., *Proc. Natl. Acad. Sci.* 87: 6378-6382 (1990); Felici, *J. Mol. Biol.* 222: 301-310 (1991); Ladner *supra*).

[0114] A compound sometimes alters expression and sometimes alters activity of a polypeptide target and may be a small molecule. Small molecules include, but are not limited to, peptides, peptidomimetics (e.g., peptoids), amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (i.e., including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

Antisense Nucleic Acid Molecules, Ribozymes, RNAi, siRNA and Modified Nucleic Acid Molecules

[0115] An “antisense” nucleic acid refers to a nucleotide sequence complementary to a “sense” nucleic acid encoding a polypeptide, e.g., complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. The antisense nucleic acid can be complementary to an entire coding strand, or to a portion thereof or a substantially identical sequence thereof. In another embodiment, the antisense nucleic acid molecule is antisense to a “noncoding region” of the coding strand of a nucleotide sequence (e.g., 5’ and 3’ untranslated regions in SEQ ID NO: 1).

[0116] An antisense nucleic acid can be designed such that it is complementary to the entire coding region of an mRNA encoded by a nucleotide sequence (e.g., SEQ ID NO: 1), and often the antisense nucleic acid is an oligonucleotide antisense to only a portion of a coding or noncoding region of the mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of the mRNA, e.g., between the -10 and +10 regions of the target gene nucleotide sequence of interest. An antisense oligonucleotide can be, for example, about 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, or more nucleotides in length. The antisense nucleic acids, which include the ribozymes described hereafter, can be designed to target a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXLI*, *CASPR4* or *GPR50* nucleotide sequence, often a variant associated with osteoarthritis, or a substantially identical sequence thereof. Among the variants, minor alleles and major alleles can be targeted, and those associated with a higher risk of osteoarthritis are often designed, tested, and administered to subjects.

[0117] An antisense nucleic acid can be constructed using chemical synthesis and enzymatic ligation reactions using standard procedures. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. Antisense nucleic acid also can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

[0118] When utilized as therapeutics, antisense nucleic acids typically are administered to a subject (e.g., by direct injection at a tissue site) or generated in situ such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a polypeptide and thereby inhibit expression of the polypeptide, for example, by inhibiting transcription and/or translation. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then are administered systemically. For

systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, for example, by linking antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. Antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. Sufficient intracellular concentrations of antisense molecules are achieved by incorporating a strong promoter, such as a pol II or pol III promoter, in the vector construct.

[0119] Antisense nucleic acid molecules sometimes are alpha-anomeric nucleic acid molecules. An alpha-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual beta-units, the strands run parallel to each other (Gaultier et al., *Nucleic Acids. Res.* 15: 6625-6641 (1987)). Antisense nucleic acid molecules can also comprise a 2'-O-methylribonucleotide (Inoue et al., *Nucleic Acids Res.* 15: 6131-6148 (1987)) or a chimeric RNA-DNA analogue (Inoue et al., *FEBS Lett.* 215: 327-330 (1987)). Antisense nucleic acids sometimes are composed of DNA or PNA or any other nucleic acid derivatives described previously.

[0120] In another embodiment, an antisense nucleic acid is a ribozyme. A ribozyme having specificity for a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleotide sequence can include one or more sequences complementary to such a nucleotide sequence, and a sequence having a known catalytic region responsible for mRNA cleavage (see e.g., U.S. Pat. No. 5,093,246 or Haselhoff and Gerlach, *Nature* 334: 585-591 (1988)). For example, a derivative of a Tetrahymena L-19 IVS RNA is sometimes utilized in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a mRNA (see e.g., Cech et al. U.S. Patent No. 4,987,071; and Cech et al. U.S. Patent No. 5,116,742). Also, target mRNA sequences can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules (see e.g., Bartel & Szostak, *Science* 261: 1411-1418 (1993)).

[0121] Osteoarthritis directed molecules include in certain embodiments nucleic acids that can form triple helix structures with a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleotide sequence, or a substantially identical sequence thereof, especially one that includes a regulatory region that controls expression of a polypeptide. Gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of a nucleotide sequence referenced herein or a substantially identical sequence (e.g., promoter and/or enhancers) to form triple helical structures that prevent transcription of a gene in target cells (see e.g., Helene, *Anticancer Drug Des.* 6(6): 569-84 (1991); Helene et al., *Ann. N.Y. Acad. Sci.* 660: 27-36 (1992); and Maher, *Bioassays* 14(12): 807-15 (1992). Potential sequences that can be targeted for triple helix formation can be increased by creating a so-called "switchback" nucleic acid molecule. Switchback molecules are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with first one strand of a duplex

and then the other, eliminating the necessity for a sizeable stretch of either purines or pyrimidines to be present on one strand of a duplex.

[0122] Osteoarthritis directed molecules include RNAi and siRNA nucleic acids. Gene expression may be inhibited by the introduction of double-stranded RNA (dsRNA), which induces potent and specific gene silencing, a phenomenon called RNA interference or RNAi. See, e.g., Fire et al., US Patent Number 6,506,559; Tuschl et al. PCT International Publication No. WO 01/75164; Kay et al. PCT International Publication No. WO 03/010180A1; or Boshier JM, Labouesse, Nat Cell Biol 2000 Feb;2(2):E31-6. This process has been improved by decreasing the size of the double-stranded RNA to 20-24 base pairs (to create small-interfering RNAs or siRNAs) that “switched off” genes in mammalian cells without initiating an acute phase response, i.e., a host defense mechanism that often results in cell death (see, e.g., Caplen et al. Proc Natl Acad Sci U S A. 2001 Aug 14;98(17):9742-7 and Elbashir et al. Methods 2002 Feb;26(2):199-213). There is increasing evidence of post-transcriptional gene silencing by RNA interference (RNAi) for inhibiting targeted expression in mammalian cells at the mRNA level, in human cells. There is additional evidence of effective methods for inhibiting the proliferation and migration of tumor cells in human patients, and for inhibiting metastatic cancer development (see, e.g., U.S. Patent Application No. US2001000993183; Caplen et al. Proc Natl Acad Sci U S A; and Abderrahmani et al. Mol Cell Biol 2001 Nov21(21):7256-67).

[0123] An “siRNA” or “RNAi” refers to a nucleic acid that forms a double stranded RNA and has the ability to reduce or inhibit expression of a gene or target gene when the siRNA is delivered to or expressed in the same cell as the gene or target gene. “siRNA” refers to short double-stranded RNA formed by the complementary strands. Complementary portions of the siRNA that hybridize to form the double stranded molecule often have substantial or complete identity to the target molecule sequence. In one embodiment, an siRNA refers to a nucleic acid that has substantial or complete identity to a target gene and forms a double stranded siRNA.

[0124] When designing the siRNA molecules, the targeted region often is selected from a given DNA sequence beginning 50 to 100 nucleotides downstream of the start codon. See, e.g., Elbashir et al., Methods 26:199-213 (2002). Initially, 5’ or 3’ UTRs and regions nearby the start codon were avoided assuming that UTR-binding proteins and/or translation initiation complexes may interfere with binding of the siRNP or RISC endonuclease complex. Sometimes regions of the target 23 nucleotides in length conforming to the sequence motif AA(N19)TT (N, an nucleotide), and regions with approximately 30% to 70% G/C-content (often about 50% G/C-content) often are selected. If no suitable sequences are found, the search often is extended using the motif NA(N21). The sequence of the sense siRNA sometimes corresponds to (N19) TT or N21 (position 3 to 23 of the 23-nt motif), respectively. In the latter case, the 3’ end of the sense siRNA often is converted to TT. The rationale for this sequence

conversion is to generate a symmetric duplex with respect to the sequence composition of the sense and antisense 3' overhangs. The antisense siRNA is synthesized as the complement to position 1 to 21 of the 23-nt motif. Because position 1 of the 23-nt motif is not recognized sequence-specifically by the antisense siRNA, the 3'-most nucleotide residue of the antisense siRNA can be chosen deliberately. However, the penultimate nucleotide of the antisense siRNA (complementary to position 2 of the 23-nt motif) often is complementary to the targeted sequence. For simplifying chemical synthesis, TT often is utilized. siRNAs corresponding to the target motif NAR(N17)YNN, where R is purine (A,G) and Y is pyrimidine (C,U), often are selected. Respective 21 nucleotide sense and antisense siRNAs often begin with a purine nucleotide and can also be expressed from pol III expression vectors without a change in targeting site. Expression of RNAs from pol III promoters often is efficient when the first transcribed nucleotide is a purine.

[0125] The sequence of the siRNA can correspond to the full length target gene, or a subsequence thereof. Often, the siRNA is about 15 to about 50 nucleotides in length (e.g., each complementary sequence of the double stranded siRNA is 15-50 nucleotides in length, and the double stranded siRNA is about 15-50 base pairs in length, sometimes about 20-30 nucleotides in length or about 20-25 nucleotides in length, e.g., 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length. The siRNA sometimes is about 21 nucleotides in length. Methods of using siRNA are well known in the art, and specific siRNA molecules may be purchased from a number of companies including Dharmacon Research, Inc.

[0126] Antisense, ribozyme, RNAi and siRNA nucleic acids can be altered to form modified nucleic acid molecules. The nucleic acids can be altered at base moieties, sugar moieties or phosphate backbone moieties to improve stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup et al., *Bioorganic & Medicinal Chemistry* 4 (1): 5-23 (1996)). As used herein, the terms "peptide nucleic acid" or "PNA" refers to a nucleic acid mimic such as a DNA mimic, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of a PNA can allow for specific hybridization to DNA and RNA under conditions of low ionic strength. Synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described, for example, in Hyrup et al., (1996) *supra* and Perry-O'Keefe et al., *Proc. Natl. Acad. Sci.* 93: 14670-675 (1996).

[0127] PNA nucleic acids can be used in prognostic, diagnostic, and therapeutic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNA nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (e.g., by PNA-directed PCR clamping); as "artificial restriction enzymes" when used in combination with other

enzymes, (e.g., S1 nucleases (Hyrup (1996) supra)); or as probes or primers for DNA sequencing or hybridization (Hyrup et al., (1996) supra; Perry-O'Keefe supra).

[0128] In other embodiments, oligonucleotides may include other appended groups such as peptides (e.g., for targeting host cell receptors in vivo), or agents facilitating transport across cell membranes (see e.g., Letsinger et al., Proc. Natl. Acad. Sci. USA 86: 6553-6556 (1989); Lemaitre et al., Proc. Natl. Acad. Sci. USA 84: 648-652 (1987); PCT Publication No. W088/09810) or the blood-brain barrier (see, e.g., PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (See, e.g., Krol et al., Bio-Techniques 6: 958-976 (1988)) or intercalating agents. (See, e.g., Zon, Pharm. Res. 5: 539-549 (1988)). To this end, the oligonucleotide may be conjugated to another molecule, (e.g., a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

[0129] Also included herein are molecular beacon oligonucleotide primer and probe molecules having one or more regions complementary to a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXLI*, *CASPR4* or *GPR50* nucleotide sequence, or a substantially identical sequence thereof, two complementary regions one having a fluorophore and one a quencher such that the molecular beacon is useful for quantifying the presence of the nucleic acid in a sample. Molecular beacon nucleic acids are described, for example, in Lizardi et al., U.S. Patent No. 5,854,033; Nazarenko et al., U.S. Patent No. 5,866,336, and Livak et al., U.S. Patent 5,876,930.

Antibodies

[0130] The term "antibody" as used herein refers to an immunoglobulin molecule or immunologically active portion thereof, i.e., an antigen-binding portion. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and F(ab')₂ fragments which can be generated by treating the antibody with an enzyme such as pepsin. An antibody sometimes is a polyclonal, monoclonal, recombinant (e.g., a chimeric or humanized), fully human, non-human (e.g., murine), or a single chain antibody. An antibody may have effector function and can fix complement, and is sometimes coupled to a toxin or imaging agent.

[0131] A full-length polypeptide or antigenic peptide fragment encoded by a nucleotide sequence referenced herein can be used as an immunogen or can be used to identify antibodies made with other immunogens, e.g., cells, membrane preparations, and the like. An antigenic peptide often includes at least 8 amino acid residues of the amino acid sequences encoded by a nucleotide sequence referenced herein, or substantially identical sequence thereof, and encompasses an epitope. Antigenic peptides sometimes include 10 or more amino acids, 15 or more amino acids, 20 or more amino acids, or 30 or

more amino acids. Hydrophilic and hydrophobic fragments of polypeptides sometimes are used as immunogens.

[0132] Epitopes encompassed by the antigenic peptide are regions located on the surface of the polypeptide (e.g., hydrophilic regions) as well as regions with high antigenicity. For example, an Emini surface probability analysis of the human polypeptide sequence can be used to indicate the regions that have a particularly high probability of being localized to the surface of the polypeptide and are thus likely to constitute surface residues useful for targeting antibody production. The antibody may bind an epitope on any domain or region on polypeptides described herein.

[0133] Also, chimeric, humanized, and completely human antibodies are useful for applications which include repeated administration to subjects. Chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, can be made using standard recombinant DNA techniques. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in Robinson et al International Application No. PCT/US86/02269; Akira, et al European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison et al European Patent Application 173,494; Neuberger et al PCT International Publication No. WO 86/01533; Cabilly et al U.S. Patent No. 4,816,567; Cabilly et al European Patent Application 125,023; Better et al., Science 240: 1041-1043 (1988); Liu et al., Proc. Natl. Acad. Sci. USA 84: 3439-3443 (1987); Liu et al., J. Immunol. 139: 3521-3526 (1987); Sun et al., Proc. Natl. Acad. Sci. USA 84: 214-218 (1987); Nishimura et al., Canc. Res. 47: 999-1005 (1987); Wood et al., Nature 314: 446-449 (1985); and Shaw et al., J. Natl. Cancer Inst. 80: 1553-1559 (1988); Morrison, S. L., Science 229: 1202-1207 (1985); Oi et al., BioTechniques 4: 214 (1986); Winter U.S. Patent 5,225,539; Jones et al., Nature 321: 552-525 (1986); Verhoeyan et al., Science 239: 1534; and Beidler et al., J. Immunol. 141: 4053-4060 (1988).

[0134] Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Such antibodies can be produced using transgenic mice that are incapable of expressing endogenous immunoglobulin heavy and light chains genes, but which can express human heavy and light chain genes. See, for example, Lonberg and Huszar, Int. Rev. Immunol. 13: 65-93 (1995); and U.S. Patent Nos. 5,625,126; 5,633,425; 5,569,825; 5,661,016; and 5,545,806. In addition, companies such as Abgenix, Inc. (Fremont, CA) and Medarex, Inc. (Princeton, NJ), can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above. Completely human antibodies that recognize a selected epitope also can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody (e.g., a murine antibody) is used to guide the selection of a completely human antibody recognizing the same epitope. This technology is described for example by Jespers et al., Bio/Technology 12: 899-903 (1994).

[0135] An antibody can be a single chain antibody. A single chain antibody (scFV) can be engineered (see, e.g., Colcher et al., Ann. N Y Acad. Sci. 880: 263-80 (1999); and Reiter, Clin. Cancer Res. 2: 245-52 (1996)). Single chain antibodies can be dimerized or multimerized to generate multivalent antibodies having specificities for different epitopes of the same target polypeptide.

[0136] Antibodies also may be selected or modified so that they exhibit reduced or no ability to bind an Fc receptor. For example, an antibody may be an isotype or subtype, fragment or other mutant, which does not support binding to an Fc receptor (e.g., it has a mutagenized or deleted Fc receptor binding region).

[0137] Also, an antibody (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1 dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thiotepa chlorambucil, melphalan, carmustine (BCNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

[0138] Antibody conjugates can be used for modifying a given biological response. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a polypeptide such as tumor necrosis factor, gamma-interferon, alpha-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors. Also, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, for example.

[0139] An antibody (e.g., monoclonal antibody) can be used to isolate target polypeptides by standard techniques, such as affinity chromatography or immunoprecipitation. Moreover, an antibody can be used to detect a target polypeptide (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the polypeptide. Antibodies can be used diagnostically to

monitor polypeptide levels in tissue as part of a clinical testing procedure, e.g., to determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (i.e., physically linking) the antibody to a detectable substance (i.e., antibody labeling). Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H . Also, an antibody can be utilized as a test molecule for determining whether it can treat osteoarthritis, and as a therapeutic for administration to a subject for treating osteoarthritis.

[0140] An antibody can be made by immunizing with a purified antigen, or a fragment thereof, e.g., a fragment described herein, a membrane associated antigen, tissues, e.g., crude tissue preparations, whole cells, preferably living cells, lysed cells, or cell fractions.

[0141] Included herein are antibodies which bind only a native polypeptide, only denatured or otherwise non-native polypeptide, or which bind both, as well as those having linear or conformational epitopes. Conformational epitopes sometimes can be identified by selecting antibodies that bind to native but not denatured polypeptide. Also featured are antibodies that specifically bind to a polypeptide variant associated with osteoarthritis.

Methods for Identifying Candidate Therapeutics for Treating Osteoarthritis

[0142] Current therapies for the treatment of osteoarthritis have limited efficacy, limited tolerability and significant mechanism-based side effects, and few of the available therapies adequately address underlying defects. Current therapeutic approaches were largely developed in the absence of defined molecular targets or even a solid understanding of disease pathogenesis. Therefore, provided are methods of identifying candidate therapeutics that target biochemical pathways related to the development of osteoarthritis.

[0143] Thus, featured herein are methods for identifying a candidate therapeutic for treating osteoarthritis. The methods comprise contacting a test molecule with a target molecule in a system. A "target molecule" as used herein refers to a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleic acid, a substantially identical nucleic acid thereof, or a fragment thereof, and an encoded polypeptide of the foregoing. The methods also comprise determining the presence or

absence of an interaction between the test molecule and the target molecule, where the presence of an interaction between the test molecule and the nucleic acid or polypeptide identifies the test molecule as a candidate osteoarthritis therapeutic. The interaction between the test molecule and the target molecule may be quantified.

[0144] Test molecules and candidate therapeutics include, but are not limited to, compounds, antisense nucleic acids, siRNA molecules, ribozymes, polypeptides or proteins encoded by a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleotide sequence, or a substantially identical sequence or fragment thereof, and immunotherapeutics (e.g., antibodies and HLA-presented polypeptide fragments). A test molecule or candidate therapeutic may act as a modulator of target molecule concentration or target molecule function in a system. A “modulator” may agonize (i.e., up-regulates) or antagonize (i.e., down-regulates) a target molecule concentration partially or completely in a system by affecting such cellular functions as DNA replication and/or DNA processing (e.g., DNA methylation or DNA repair), RNA transcription and/or RNA processing (e.g., removal of intronic sequences and/or translocation of spliced mRNA from the nucleus), polypeptide production (e.g., translation of the polypeptide from mRNA), and/or polypeptide post-translational modification (e.g., glycosylation, phosphorylation, and proteolysis of pro-polypeptides). A modulator may also agonize or antagonize a biological function of a target molecule partially or completely, where the function may include adopting a certain structural conformation, interacting with one or more binding partners, ligand binding, catalysis (e.g., phosphorylation, dephosphorylation, hydrolysis, methylation, and isomerization), and an effect upon a cellular event (e.g., effecting progression of osteoarthritis). Any modulator may be utilized, such as a peptidyl arginine deiminase modulator (e.g., *PADI2* likely is a peptidyl arginine deiminase) described in WO-09851784 and WO0244360A2 or an apolipoprotein (e.g., *APOB* includes an apolipoprotein domain) modulatory compound (e.g., WO-2004017969, WO-03002533, US 6,369,075, WO-02098839, WO-02098871, WO-00177077, WO-00153260, WO-00105767), antibody (e.g., WO-9600903A1, US 6,309,844 and US 5,330,910) or antisense molecule (e.g., WO03011887A2 and WO03097662A1).

[0145] As used herein, the term “system” refers to a cell free *in vitro* environment and a cell-based environment such as a collection of cells, a tissue, an organ, or an organism. A system is “contacted” with a test molecule in a variety of manners, including adding molecules in solution and allowing them to interact with one another by diffusion, cell injection, and any administration routes in an animal. As used herein, the term “interaction” refers to an effect of a test molecule on test molecule, where the effect sometimes is binding between the test molecule and the target molecule, and sometimes is an observable change in cells, tissue, or organism.

[0146] There are many standard methods for detecting the presence or absence of interaction between a test molecule and a target molecule. For example, titrametric, acidimetric, radiometric, NMR, monolayer, polarographic, spectrophotometric, fluorescent, and ESR assays probative of a target molecule interaction may be utilized. Examples of G protein-coupled receptor assays are known, for example, and are described in WO-0242461 and WO-04013285.

[0147] Test molecule/target molecule interactions can be detected and/or quantified using assays known in the art. For example, an interaction can be determined by labeling the test molecule and/or the target molecule, where the label is covalently or non-covalently attached to the test molecule or target molecule. The label is sometimes a radioactive molecule such as ^{125}I , ^{131}I , ^{35}S or ^3H , which can be detected by direct counting of radioemission or by scintillation counting. Also, enzymatic labels such as horseradish peroxidase, alkaline phosphatase, or luciferase may be utilized where the enzymatic label can be detected by determining conversion of an appropriate substrate to product. In addition, presence or absence of an interaction can be determined without labeling. For example, a microphysiometer (*e.g.*, Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indication of an interaction between a test molecule and target molecule (McConnell, H. M. *et al.*, *Science* 257: 1906-1912 (1992)).

[0148] In cell-based systems, cells typically include a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXLI*, *CASPR4* or *GPR50* nucleic acid, an encoded polypeptide, or substantially identical nucleic acid or polypeptide thereof, and are often of mammalian origin, although the cell can be of any origin. Whole cells, cell homogenates, and cell fractions (*e.g.*, cell membrane fractions) can be subjected to analysis. Where interactions between a test molecule with a target polypeptide are monitored, soluble and/or membrane bound forms of the polypeptide may be utilized. Where membrane-bound forms of the polypeptide are used, it may be desirable to utilize a solubilizing agent. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton[®] X-100, Triton[®] X-114, Thesit[®], Isotridecypoly(ethylene glycol ether)_n, 3-[(3-cholamidopropyl)dimethylamminio]-1-propane sulfonate (CHAPS), 3-[(3-cholamidopropyl)dimethylamminio]-2-hydroxy-1-propane sulfonate (CHAPSO), or N-dodecyl-N,N-dimethyl-3-ammonio-1-propane sulfonate.

[0149] An interaction between a test molecule and target molecule also can be detected by monitoring fluorescence energy transfer (FET) (*see, e.g.*, Lakowicz *et al.*, U.S. Patent No. 5,631,169; Stavrianopoulos *et al.* U.S. Patent No. 4,868,103). A fluorophore label on a first, "donor" molecule is selected such that its emitted fluorescent energy will be absorbed by a fluorescent label on a second,

“acceptor” molecule, which in turn is able to fluoresce due to the absorbed energy. Alternately, the “donor” polypeptide molecule may simply utilize the natural fluorescent energy of tryptophan residues. Labels are chosen that emit different wavelengths of light, such that the “acceptor” molecule label may be differentiated from that of the “donor”. Since the efficiency of energy transfer between the labels is related to the distance separating the molecules, the spatial relationship between the molecules can be assessed. In a situation in which binding occurs between the molecules, the fluorescent emission of the “acceptor” molecule label in the assay should be maximal. An FET binding event can be conveniently measured through standard fluorometric detection means well known in the art (*e.g.*, using a fluorimeter).

[0150] In another embodiment, determining the presence or absence of an interaction between a test molecule and a target molecule can be effected by monitoring surface plasmon resonance (*see, e.g.*, Sjolander & Urbanicz, *Anal. Chem.* 63: 2338-2345 (1991) and Szabo *et al.*, *Curr. Opin. Struct. Biol.* 5: 699-705 (1995)). “Surface plasmon resonance” or “biomolecular interaction analysis (BIA)” can be utilized to detect biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore). Changes in the mass at the binding surface (indicative of a binding event) result in alterations of the refractive index of light near the surface (the optical phenomenon of surface plasmon resonance (SPR)), resulting in a detectable signal which can be used as an indication of real-time reactions between biological molecules.

[0151] In another embodiment, the target molecule or test molecules are anchored to a solid phase, facilitating the detection of target molecule/test molecule complexes and separation of the complexes from free, uncomplexed molecules. The target molecule or test molecule is immobilized to the solid support. In an embodiment, the target molecule is anchored to a solid surface, and the test molecule, which is not anchored, can be labeled, either directly or indirectly, with detectable labels discussed herein.

[0152] It may be desirable to immobilize a target molecule, an anti-target molecule antibody, and/or test molecules to facilitate separation of target molecule/test molecule complexes from uncomplexed forms, as well as to accommodate automation of the assay. The attachment between a test molecule and/or target molecule and the solid support may be covalent or non-covalent (*see, e.g.*, U.S. Patent No. 6,022,688 for non-covalent attachments). The solid support may be one or more surfaces of the system, such as one or more surfaces in each well of a microtiter plate, a surface of a silicon wafer, a surface of a bead (*see, e.g.*, Lam, *Nature* 354: 82-84 (1991)) that is optionally linked to another solid support, or a channel in a microfluidic device, for example. Types of solid supports, linker molecules for covalent and non-covalent attachments to solid supports, and methods for immobilizing nucleic acids and other molecules to solid supports are well known (*see, e.g.*, U.S. Patent Nos. 6,261,776; 5,900,481; 6,133,436; and 6,022,688; and WIPO publication WO 01/18234).

[0153] In an embodiment, target molecule may be immobilized to surfaces via biotin and streptavidin. For example, biotinylated target polypeptide can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). In another embodiment, a target polypeptide can be prepared as a fusion polypeptide. For example, glutathione-S-transferase/target polypeptide fusion can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivitized microtiter plates, which are then combined with a test molecule under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, or the matrix is immobilized in the case of beads, and complex formation is determined directly or indirectly as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of target molecule binding or activity is determined using standard techniques.

[0154] In an embodiment, the non-immobilized component is added to the coated surface containing the anchored component. After the reaction is complete, unreacted components are removed (*e.g.*, by washing) under conditions such that a significant percentage of complexes formed will remain immobilized to the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of manners. Where the previously non-immobilized component is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the previously non-immobilized component is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface, *e.g.*, by adding a labeled antibody specific for the immobilized component, where the antibody, in turn, can be directly labeled or indirectly labeled with, *e.g.*, a labeled anti-Ig antibody.

[0155] In another embodiment, an assay is performed utilizing antibodies that specifically bind target molecule or test molecule but do not interfere with binding of the target molecule to the test molecule. Such antibodies can be derivitized to a solid support, and unbound target molecule may be immobilized by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the target molecule.

[0156] Cell free assays also can be conducted in a liquid phase. In such an assay, reaction products are separated from unreacted components, by any of a number of standard techniques, including but not limited to: differential centrifugation (*see, e.g.*, Rivas, G., and Minton, *Trends Biochem Sci Aug;18(8): 284-7 (1993)*); chromatography (gel filtration chromatography, ion-exchange chromatography); electrophoresis (*see, e.g.*, Ausubel *et al.*, eds. *Current Protocols in Molecular Biology*, J. Wiley: New

York (1999)); and immunoprecipitation (*see, e.g., Ausubel et al., eds., supra*). Media and chromatographic techniques are known to one skilled in the art (*see, e.g., Heegaard, J. Mol. Recognit. Winter; 11(1-6): 141-8 (1998); Hage & Tweed, J. Chromatogr. B Biomed. Sci. Appl. Oct 10; 699 (1-2): 499-525 (1997)*). Further, fluorescence energy transfer may also be conveniently utilized, as described herein, to detect binding without further purification of the complex from solution.

[0157] In another embodiment, modulators of target molecule expression are identified. For example, a cell or cell free mixture is contacted with a candidate compound and the expression of target mRNA or target polypeptide is evaluated relative to the level of expression of target mRNA or target polypeptide in the absence of the candidate compound. When expression of target mRNA or target polypeptide is greater in the presence of the candidate compound than in its absence, the candidate compound is identified as an agonist of target mRNA or target polypeptide expression. Alternatively, when expression of target mRNA or target polypeptide is less (*e.g., less with statistical significance*) in the presence of the candidate compound than in its absence, the candidate compound is identified as an antagonist or inhibitor of target mRNA or target polypeptide expression. The level of target mRNA or target polypeptide expression can be determined by methods described herein.

[0158] In another embodiment, binding partners that interact with a target molecule are detected. The target molecules can interact with one or more cellular or extracellular macromolecules, such as polypeptides *in vivo*, and these interacting molecules are referred to herein as “binding partners.” Binding partners can agonize or antagonize target molecule biological activity. Also, test molecules that agonize or antagonize interactions between target molecules and binding partners can be useful as therapeutic molecules as they can up-regulate or down-regulated target molecule activity *in vivo* and thereby treat osteoarthritis.

[0159] Binding partners of target molecules can be identified by methods known in the art. For example, binding partners may be identified by lysing cells and analyzing cell lysates by electrophoretic techniques. Alternatively, a two-hybrid assay or three-hybrid assay can be utilized (*see, e.g., U.S. Patent No. 5,283,317; Zervos et al., Cell 72:223-232 (1993); Madura et al., J. Biol. Chem. 268: 12046-12054 (1993); Bartel et al., Biotechniques 14: 920-924 (1993); Iwabuchi et al., Oncogene 8: 1693-1696 (1993); and Brent WO94/10300*). A two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. The assay often utilizes two different DNA constructs. In one construct, a *PADI2, APOB, IL1RL2, WASPIP, BVES, PELI2, LOXLI, CASPR4* or *GPR50* nucleic acid (sometimes referred to as the “bait”) is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g., GAL-4*). In another construct, a DNA sequence from a library of DNA sequences that encodes a potential binding partner (sometimes referred to as the “prey”) is fused to a gene that encodes an activation domain of the known transcription factor.

Sometimes, a *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4* or *GPR50* nucleic acid can be fused to the activation domain. If the “bait” and the “prey” molecules interact *in vivo*, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, *LacZ*) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to identify the potential binding partner.

[0160] In an embodiment for identifying test molecules that antagonize or agonize complex formation between target molecules and binding partners, a reaction mixture containing the target molecule and the binding partner is prepared, under conditions and for a time sufficient to allow complex formation. The reaction mixture often is provided in the presence or absence of the test molecule. The test molecule can be included initially in the reaction mixture, or can be added at a time subsequent to the addition of the target molecule and its binding partner. Control reaction mixtures are incubated without the test molecule or with a placebo. Formation of any complexes between the target molecule and the binding partner then is detected. Decreased formation of a complex in the reaction mixture containing test molecule as compared to in a control reaction mixture indicates that the molecule antagonizes target molecule/binding partner complex formation. Alternatively, increased formation of a complex in the reaction mixture containing test molecule as compared to in a control reaction mixture indicates that the molecule agonizes target molecule/binding partner complex formation. In another embodiment, complex formation of target molecule/binding partner can be compared to complex formation of mutant target molecule/binding partner (*e.g.*, amino acid modifications in a target polypeptide). Such a comparison can be important in those cases where it is desirable to identify test molecules that modulate interactions of mutant but not non-mutated target gene products.

[0161] The assays can be conducted in a heterogeneous or homogeneous format. In heterogeneous assays, target molecule and/or the binding partner are immobilized to a solid phase, and complexes are detected on the solid phase at the end of the reaction. In homogeneous assays, the entire reaction is carried out in a liquid phase. In either approach, the order of addition of reactants can be varied to obtain different information about the molecules being tested. For example, test compounds that agonize target molecule/binding partner interactions can be identified by conducting the reaction in the presence of the test molecule in a competition format. Alternatively, test molecules that agonize preformed complexes, *e.g.*, molecules with higher binding constants that displace one of the components from the complex, can be tested by adding the test compound to the reaction mixture after complexes have been formed.

[0162] In a heterogeneous assay embodiment, the target molecule or the binding partner is anchored onto a solid surface (*e.g.*, a microtiter plate), while the non-anchored species is labeled, either directly or

indirectly. The anchored molecule can be immobilized by non-covalent or covalent attachments. Alternatively, an immobilized antibody specific for the molecule to be anchored can be used to anchor the molecule to the solid surface. The partner of the immobilized species is exposed to the coated surface with or without the test molecule. After the reaction is complete, unreacted components are removed (*e.g.*, by washing) such that a significant portion of any complexes formed will remain immobilized on the solid surface. Where the non-immobilized species is pre-labeled, the detection of label immobilized on the surface is indicative of complex. Where the non-immobilized species is not pre-labeled, an indirect label can be used to detect complexes anchored to the surface; *e.g.*, by using a labeled antibody specific for the initially non-immobilized species. Depending upon the order of addition of reaction components, test compounds that inhibit complex formation or that disrupt preformed complexes can be detected.

[0163] In another embodiment, the reaction can be conducted in a liquid phase in the presence or absence of test molecule, where the reaction products are separated from unreacted components, and the complexes are detected (*e.g.*, using an immobilized antibody specific for one of the binding components to anchor any complexes formed in solution, and a labeled antibody specific for the other partner to detect anchored complexes). Again, depending upon the order of addition of reactants to the liquid phase, test compounds that inhibit complex or that disrupt preformed complexes can be identified.

[0164] In an alternate embodiment, a homogeneous assay can be utilized. For example, a preformed complex of the target gene product and the interactive cellular or extracellular binding partner product is prepared. One or both of the target molecule or binding partner is labeled, and the signal generated by the label(s) is quenched upon complex formation (*e.g.*, U.S. Patent No. 4,109,496 that utilizes this approach for immunoassays). Addition of a test molecule that competes with and displaces one of the species from the preformed complex will result in the generation of a signal above background. In this way, test substances that disrupt target molecule/binding partner complexes can be identified.

[0165] Candidate therapeutics for treating osteoarthritis are identified from a group of test molecules that interact with a target molecule. Test molecules are normally ranked according to the degree with which they modulate (*e.g.*, agonize or antagonize) a function associated with the target molecule (*e.g.*, DNA replication and/or processing, RNA transcription and/or processing, polypeptide production and/or processing, and/or biological function/activity), and then top ranking modulators are selected. Also, pharmacogenomic information described herein can determine the rank of a modulator. The top 10% of ranked test molecules often are selected for further testing as candidate therapeutics, and sometimes the top 15%, 20%, or 25% of ranked test molecules are selected for further testing as candidate therapeutics. Candidate therapeutics typically are formulated for administration to a subject.

Therapeutic Formulations

[0166] Formulations and pharmaceutical compositions typically include in combination with a pharmaceutically acceptable carrier one or more target molecule modulators. The modulator often is a test molecule identified as having an interaction with a target molecule by a screening method described above. The modulator may be a compound, an antisense nucleic acid, a ribozyme, an antibody, or a binding partner. Also, formulations may comprise a target polypeptide or fragment thereof in combination with a pharmaceutically acceptable carrier.

[0167] As used herein, the term “pharmaceutically acceptable carrier” includes solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Supplementary active compounds can also be incorporated into the compositions. Pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

[0168] A pharmaceutical composition typically is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerin, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[0169] Oral compositions generally include an inert diluent or an edible carrier. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules, *e.g.*, gelatin capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[0170] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[0171] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0172] For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

[0173] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally

known in the art. Molecules can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[0174] In one embodiment, active molecules are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. Materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

[0175] It is advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

[0176] Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Molecules which exhibit high therapeutic indices are preferred. While molecules that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

[0177] The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such molecules lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any molecules used in the methods described herein, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be

used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

[0178] As defined herein, a therapeutically effective amount of protein or polypeptide (*i.e.*, an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, sometimes about 0.01 to 25 mg/kg body weight, often about 0.1 to 20 mg/kg body weight, and more often about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The protein or polypeptide can be administered one time per week for between about 1 to 10 weeks, sometimes between 2 to 8 weeks, often between about 3 to 7 weeks, and more often for about 4, 5, or 6 weeks. The skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide, or antibody can include a single treatment or, preferably, can include a series of treatments.

[0179] With regard to polypeptide formulations, featured herein is a method for treating osteoarthritis in a subject, which comprises contacting one or more cells in the subject with a first polypeptide, where the subject comprises a second polypeptide having one or more polymorphic variations associated with cancer, and where the first polypeptide comprises fewer polymorphic variations associated with cancer than the second polypeptide. The first and second polypeptides are encoded by a nucleic acid which comprises a nucleotide sequence in SEQ ID NO: 1-17; a nucleotide sequence which encodes a polypeptide consisting of an amino acid sequence encoded by a nucleotide sequence referenced in SEQ ID NO: 1-17; a nucleotide sequence which encodes a polypeptide that is 90% or more identical to an amino acid sequence encoded by a nucleotide sequence of SEQ ID NO: 1-17 and a nucleotide sequence 90% or more identical to a nucleotide sequence in SEQ ID NO: 1-17. The subject often is a human.

[0180] For antibodies, a dosage of 0.1 mg/kg of body weight (generally 10 mg/kg to 20 mg/kg) is often utilized. If the antibody is to act in the brain, a dosage of 50 mg/kg to 100 mg/kg is often appropriate. Generally, partially human antibodies and fully human antibodies have a longer half-life within the human body than other antibodies. Accordingly, lower dosages and less frequent administration is often possible. Modifications such as lipidation can be used to stabilize antibodies and to enhance uptake and tissue penetration (*e.g.*, into the brain). A method for lipidation of antibodies is described by Cruikshank *et al.*, *J. Acquired Immune Deficiency Syndromes and Human Retrovirology* 14:193 (1997).

[0181] Antibody conjugates can be used for modifying a given biological response, the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety

may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a polypeptide such as tumor necrosis factor, alpha-interferon, beta-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors. Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

[0182] For compounds, exemplary doses include milligram or microgram amounts of the compound per kilogram of subject or sample weight, for example, about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram. It is understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated. When one or more of these small molecules is to be administered to an animal (*e.g.*, a human) in order to modulate expression or activity of a polypeptide or nucleic acid described herein, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

[0183] With regard to nucleic acid formulations, gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (*see, e.g.*, U.S. Patent 5,328,470) or by stereotactic injection (*see e.g.*, Chen *et al.*, (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-3057). Pharmaceutical preparations of gene therapy vectors can include a gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells (*e.g.*, retroviral vectors) the pharmaceutical preparation can include one or more cells which produce the gene delivery system. Examples of gene delivery vectors are described herein.

Therapeutic Methods

[0184] A therapeutic formulation described above can be administered to a subject in need of a therapeutic for inducing a desired biological response.. Therapeutic formulations can be administered by any of the paths described herein. With regard to both prophylactic and therapeutic methods of

treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from pharmacogenomic analyses described herein.

[0185] As used herein, the term “treatment” is defined as the application or administration of a therapeutic formulation to a subject, or application or administration of a therapeutic agent to an isolated tissue or cell line from a subject with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect osteoarthritis, symptoms of osteoarthritis or a predisposition towards osteoarthritis. A therapeutic formulation includes, but is not limited to, small molecules, peptides, antibodies, ribozymes and antisense oligonucleotides. Administration of a therapeutic formulation can occur prior to the manifestation of symptoms characteristic of osteoarthritis, such that osteoarthritis is prevented or delayed in its progression. The appropriate therapeutic composition can be determined based on screening assays described herein.

[0186] As discussed, successful treatment of osteoarthritis can be brought about by techniques that serve to agonize target molecule expression or function, or alternatively, antagonize target molecule expression or function. These techniques include administration of modulators that include, but are not limited to, small organic or inorganic molecules; antibodies (including, for example, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain antibodies, and Fab, F(ab')₂ and Fab expression library fragments, scFV molecules, and epitope-binding fragments thereof); and peptides, phosphopeptides, or polypeptides.

[0187] Further, antisense and ribozyme molecules that inhibit expression of the target gene can also be used to reduce the level of target gene expression, thus effectively reducing the level of target gene activity. Still further, triple helix molecules can be utilized in reducing the level of target gene activity. Antisense, ribozyme and triple helix molecules are discussed above. It is possible that the use of antisense, ribozyme, and/or triple helix molecules to reduce or inhibit mutant gene expression can also reduce or inhibit the transcription (triple helix) and/or translation (antisense, ribozyme) of mRNA produced by normal target gene alleles, such that the concentration of normal target gene product present can be lower than is necessary for a normal phenotype. In such cases, nucleic acid molecules that encode and express target gene polypeptides exhibiting normal target gene activity can be introduced into cells via gene therapy method. Alternatively, in instances in that the target gene encodes an extracellular polypeptide, it can be preferable to co-administer normal target gene polypeptide into the cell or tissue in order to maintain the requisite level of cellular or tissue target gene activity.

[0188] Another method by which nucleic acid molecules may be utilized in treating or preventing osteoarthritis is use of aptamer molecules specific for target molecules. Aptamers are nucleic acid molecules having a tertiary structure which permits them to specifically bind to ligands (*see, e.g.,*

Osborne, *et al.*, *Curr. Opin. Chem. Biol.* 1(1): 5-9 (1997); and Patel, D. J., *Curr. Opin. Chem. Biol. Jun*; 1(1): 32-46 (1997)).

[0189] Yet another method of utilizing nucleic acid molecules for osteoarthritis treatment is gene therapy, which can also be referred to as allele therapy. Provided herein is a gene therapy method for treating osteoarthritis in a subject, which comprises contacting one or more cells in the subject or from the subject with a nucleic acid having a first nucleotide sequence (e.g., the first nucleotide sequence is identical to or substantially identical to a nucleotide sequence of SEQ ID NO: 1-17). Genomic DNA in the subject comprises a second nucleotide sequence having one or more polymorphic variations associated with osteoarthritis (e.g., the second nucleotide sequence is identical to or substantially identical to a nucleotide sequence of SEQ ID NO: 1-6). The first and second nucleotide sequences typically are substantially identical to one another, and the first nucleotide sequence comprises fewer polymorphic variations associated with osteoarthritis than the second nucleotide sequence. The first nucleotide sequence may comprise a gene sequence that encodes a full-length polypeptide or a fragment thereof. The subject is often a human. Allele therapy methods often are utilized in conjunction with a method of first determining whether a subject has genomic DNA that includes polymorphic variants associated with osteoarthritis.

[0190] In another allele therapy embodiment, provided herein is a method which comprises contacting one or more cells in the subject or from the subject with a polypeptide encoded by a nucleic acid having a first nucleotide sequence (e.g., the first nucleotide sequence is identical to or substantially identical to the nucleotide sequence of SEQ ID NO: 1-17). Genomic DNA in the subject comprises a second nucleotide sequence having one or more polymorphic variations associated with osteoarthritis (e.g., the second nucleotide sequence is identical to or substantially identical to a nucleotide sequence of SEQ ID NO: 1-6). The first and second nucleotide sequences typically are substantially identical to one another, and the first nucleotide sequence comprises fewer polymorphic variations associated with osteoarthritis than the second nucleotide sequence. The first nucleotide sequence may comprise a gene sequence that encodes a full-length polypeptide or a fragment thereof. The subject is often a human.

[0191] For antibody-based therapies, antibodies can be generated that are both specific for target molecules and that reduce target molecule activity. Such antibodies may be administered in instances where antagonizing a target molecule function is appropriate for the treatment of osteoarthritis.

[0192] In circumstances where stimulating antibody production in an animal or a human subject by injection with a target molecule is harmful to the subject, it is possible to generate an immune response against the target molecule by use of anti-idiotypic antibodies (*see, e.g.*, Herlyn, *Ann. Med.*; 31(1): 66-78 (1999); and Bhattacharya-Chatterjee & Foon, *Cancer Treat. Res.*; 94: 51-68 (1998)). Introducing an anti-idiotypic antibody to a mammal or human subject often stimulates production of anti-anti-idiotypic

antibodies, which typically are specific to the target molecule. Vaccines directed to osteoarthritis also may be generated in this fashion.

[0193] In instances where the target molecule is intracellular and whole antibodies are used, internalizing antibodies may be preferred. Lipofectin or liposomes can be used to deliver the antibody or a fragment of the Fab region that binds to the target antigen into cells. Where fragments of the antibody are used, the smallest inhibitory fragment that binds to the target antigen is preferred. For example, peptides having an amino acid sequence corresponding to the Fv region of the antibody can be used. Alternatively, single chain neutralizing antibodies that bind to intracellular target antigens can also be administered. Such single chain antibodies can be administered, for example, by expressing nucleotide sequences encoding single-chain antibodies within the target cell population (*see, e.g., Marasco et al., Proc. Natl. Acad. Sci. USA 90: 7889-7893 (1993)*).

[0194] Modulators can be administered to a patient at therapeutically effective doses to treat osteoarthritis. A therapeutically effective dose refers to an amount of the modulator sufficient to result in amelioration of symptoms of osteoarthritis. Toxicity and therapeutic efficacy of modulators can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Modulators that exhibit large therapeutic indices are preferred. While modulators that exhibit toxic side effects can be used, care should be taken to design a delivery system that targets such molecules to the site of affected tissue in order to minimize potential damage to uninfected cells, thereby reducing side effects.

[0195] Data obtained from cell culture assays and animal studies can be used in formulating a range of dosages for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the methods described herein, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (*i.e.*, the concentration of the test compound that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

[0196] Another example of effective dose determination for an individual is the ability to directly assay levels of “free” and “bound” compound in the serum of the test subject. Such assays may utilize antibody mimics and/or “biosensors” that have been created through molecular imprinting techniques.

Molecules that modulate target molecule activity are used as a template, or “imprinting molecule”, to spatially organize polymerizable monomers prior to their polymerization with catalytic reagents. The subsequent removal of the imprinted molecule leaves a polymer matrix which contains a repeated “negative image” of the compound and is able to selectively rebind the molecule under biological assay conditions. A detailed review of this technique can be seen in Ansell *et al.*, *Current Opinion in Biotechnology* 7: 89-94 (1996) and in Shea, *Trends in Polymer Science* 2: 166-173 (1994). Such “imprinted” affinity matrixes are amenable to ligand-binding assays, whereby the immobilized monoclonal antibody component is replaced by an appropriately imprinted matrix. An example of the use of such matrixes in this way can be seen in Vlatakis, *et al.*, *Nature* 361: 645-647 (1993). Through the use of isotope-labeling, the “free” concentration of compound which modulates target molecule expression or activity readily can be monitored and used in calculations of IC_{50} . Such “imprinted” affinity matrixes can also be designed to include fluorescent groups whose photon-emitting properties measurably change upon local and selective binding of target compound. These changes readily can be assayed in real time using appropriate fiberoptic devices, in turn allowing the dose in a test subject to be quickly optimized based on its individual IC_{50} . An example of such a “biosensor” is discussed in Kriz *et al.*, *Analytical Chemistry* 67: 2142-2144 (1995).

[0197] The examples set forth below are intended to illustrate but not limit the invention.

Examples

[0198] In the following studies a group of subjects was selected according to specific parameters relating to osteoarthritis. Nucleic acid samples obtained from individuals in the study group were subjected to genetic analysis, which identified associations between osteoarthritis and polymorphisms in the following genes: *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4*, and *GPR50* (herein referred to as “targets”). The polymorphisms were genotyped again in two replication cohorts consisting of individuals selected for OA. In addition, SNPs proximal to the incident polymorphisms were identified and allelotyped in OA case and control pools. Methods are described for producing *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4*, and *GPR50* polypeptide and polypeptide variants thereof *in vitro* or *in vivo*; *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4*, and *GPR50* nucleic acids or polypeptides and variants thereof are utilized for screening test molecules for those that interact with *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4*, and *GPR50* molecules. Test molecules identified as interactors with *PADI2*, *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *PELI2*, *LOXL1*, *CASPR4*, and *GPR50* molecules and variants thereof are further screened *in vivo* to determine whether they treat osteoarthritis.

Example 1
Samples and Pooling Strategies

Sample Selection

[0199] Blood samples were collected from individuals diagnosed with knee osteoarthritis, which were referred to as case samples. Also, blood samples were collected from individuals not diagnosed with knee osteoarthritis as gender and age-matched controls. A database was created that listed all phenotypic trait information gathered from individuals for each case and control sample. Genomic DNA was extracted from each of the blood samples for genetic analyses.

DNA Extraction from Blood Samples

[0200] Six to ten milliliters of whole blood was transferred to a 50 ml tube containing 27 ml of red cell lysis solution (RCL). The tube was inverted until the contents were mixed. Each tube was incubated for 10 minutes at room temperature and inverted once during the incubation. The tubes were then centrifuged for 20 minutes at 3000 x g and the supernatant was carefully poured off. 100-200 µl of residual liquid was left in the tube and was pipetted repeatedly to resuspend the pellet in the residual supernatant. White cell lysis solution (WCL) was added to the tube and pipetted repeatedly until completely mixed. While no incubation was normally required, the solution was incubated at 37°C or room temperature if cell clumps were visible after mixing until the solution was homogeneous. 2 ml of protein precipitation was added to the cell lysate. The mixtures were vortexed vigorously at high speed for 20 sec to mix the protein precipitation solution uniformly with the cell lysate, and then centrifuged for 10 minutes at 3000 x g. The supernatant containing the DNA was then poured into a clean 15 ml tube, which contained 7 ml of 100% isopropanol. The samples were mixed by inverting the tubes gently until white threads of DNA were visible. Samples were centrifuged for 3 minutes at 2000 x g and the DNA was visible as a small white pellet. The supernatant was decanted and 5 ml of 70% ethanol was added to each tube. Each tube was inverted several times to wash the DNA pellet, and then centrifuged for 1 minute at 2000 x g. The ethanol was decanted and each tube was drained on clean absorbent paper. The DNA was dried in the tube by inversion for 10 minutes, and then 1000 µl of 1X TE was added. The size of each sample was estimated, and less TE buffer was added during the following DNA hydration step if the sample was smaller. The DNA was allowed to rehydrate overnight at room temperature, and DNA samples were stored at 2-8°C.

[0201] DNA was quantified by placing samples on a hematology mixer for at least 1 hour. DNA was serially diluted (typically 1:80, 1:160, 1:320, and 1:640 dilutions) so that it would be within the measurable range of standards. 125 µl of diluted DNA was transferred to a clear U-bottom microtitre

plate, and 125 µl of 1X TE buffer was transferred into each well using a multichannel pipette. The DNA and 1X TE were mixed by repeated pipetting at least 15 times, and then the plates were sealed. 50 µl of diluted DNA was added to wells A5-H12 of a black flat bottom microtitre plate. Standards were inverted six times to mix them, and then 50 µl of 1X TE buffer was pipetted into well A1, 1000 ng/ml of standard was pipetted into well A2, 500 ng/ml of standard was pipetted into well A3, and 250 ng/ml of standard was pipetted into well A4. PicoGreen (Molecular Probes, Eugene, Oregon) was thawed and freshly diluted 1:200 according to the number of plates that were being measured. PicoGreen was vortexed and then 50µl was pipetted into all wells of the black plate with the diluted DNA. DNA and PicoGreen were mixed by pipetting repeatedly at least 10 times with the multichannel pipette. The plate was placed into a Fluoroskan Ascent Machine (microplate fluorometer produced by Labsystems) and the samples were allowed to incubate for 3 minutes before the machine was run using filter pairs 485 nm excitation and 538 nm emission wavelengths. Samples having measured DNA concentrations of greater than 450 ng/µl were re-measured for conformation. Samples having measured DNA concentrations of 20 ng/µl or less were re-measured for confirmation.

Pooling Strategies – Discovery Cohort

[0202] Samples were derived from the Nottingham knee OA family study (UK) where index cases were identified through a knee replacement registry. Siblings were approached and assessed with knee x-rays and assigned status as affected or unaffected. In all 1,157 individuals were available. In order to create same-sex pools of appropriate sizes, 335 unrelated female individuals with OA from the Nottingham OA sample were selected for the case pool. The control pool was made up of unrelated female individuals from the St. Thomas twin study (England) with normal knee x-rays and without other indications of OA, regardless of anatomical location, as well as lacking family history of OA. The St. Thomas twin study consists of Caucasian, female participants from the St. Thomas' Hospital, London, adult-twin registry, which is a voluntary registry of >4,000 twin pairs ranging from 18 to 76 years of age. The female case samples and female control samples are described further in Table 1 below.

[0203] A select set of samples from each group were utilized to generate pools, and one pool was created for each group. Each individual sample in a pool was represented by an equal amount of genomic DNA. For example, where 25 ng of genomic DNA was utilized in each PCR reaction and there were 200 individuals in each pool, each individual would provide 125 pg of genomic DNA. Inclusion or exclusion of samples for a pool was based upon the following criteria: the sample was derived from an individual characterized as Caucasian; the sample was derived from an individual of British paternal and maternal descent; case samples were derived from individuals diagnosed with specific knee osteoarthritis

(OA) and were recruited from an OA knee replacement clinic. Control samples were derived from individuals free of OA, family history of OA, and rheumatoid arthritis. Also, sufficient genomic DNA was extracted from each blood sample for all allelotyping and genotyping reactions performed during the study. Phenotype information from each individual was collected and included age of the individual, gender, family history of OA, general medical information (e.g., height, weight, thyroid disease, diabetes, psoriasis, hysterectomy), joint history (previous and current symptoms, joint-related operations, age at onset of symptoms, date of primary diagnosis, age of individual as of primary diagnosis and order of involvement), and knee-related findings (crepitus, restricted passive movement, bony swelling/deformity). Additional knee information included knee history, current symptoms, any major knee injury, menisectomy, knee replacement surgery, age of surgery, and treatment history (including hormone replace therapy (HRT)). Samples that met these criteria were added to appropriate pools based on disease status.

[0204] The selection process yielded the pools set forth in Table 1, which were used in the studies that follow:

TABLE 1

	Female case	Female control
Pool size (Number)	335	335
Pool Criteria (ex: case/control)	control	case
Mean Age (ex: years)	57.21	69.95

Example 2

Association of Polymorphic Variants with Osteoarthritis

[0205] A whole-genome screen was performed to identify particular SNPs associated with occurrence of osteoarthritis. As described in Example 1, two sets of samples were utilized, which included samples from female individuals having knee osteoarthritis (osteoarthritis cases), and samples from female individuals not having knee osteoarthritis (female controls). The initial screen of each pool was performed in an allelotyping study, in which certain samples in each group were pooled. By pooling DNA from each group, an allele frequency for each SNP in each group was calculated. These allele frequencies were then compared to one another. Particular SNPs were considered as being associated with osteoarthritis when allele frequency differences calculated between case and control pools were statistically significant. SNP disease association results obtained from the allelotyping study were then validated by genotyping each associated SNP across all samples from each pool. The results of the

genotyping then were analyzed, allele frequencies for each group were calculated from the individual genotyping results, and a p-value was calculated to determine whether the case and control groups had statistically significant differences in allele frequencies for a particular SNP. When the genotyping results agreed with the original allelotyping results, the SNP disease association was considered validated at the genetic level.

SNP Panel Used for Genetic Analyses

[0206] A whole-genome SNP screen began with an initial screen of approximately 25,000 SNPs over each set of disease and control samples using a pooling approach. The pools studied in the screen are described in Example 1. The SNPs analyzed in this study were part of a set of 25,488 SNPs confirmed as being statistically polymorphic as each is characterized as having a minor allele frequency of greater than 10%. The SNPs in the set reside in genes or in close proximity to genes, and many reside in gene exons. Specifically, SNPs in the set are located in exons, introns, and within 5,000 base-pairs upstream of a transcription start site of a gene. In addition, SNPs were selected according to the following criteria: they are located in ESTs; they are located in Locuslink or Ensembl genes; and they are located in Genomatix promoter predictions. SNPs in the set were also selected on the basis of even spacing across the genome, as depicted in Table 2.

[0207] A case-control study design using a whole genome association strategy involving approximately 28,000 single nucleotide polymorphisms (SNPs) was employed. Approximately 25,000 SNPs were evenly spaced in gene-based regions of the human genome with a median inter-marker distance of about 40,000 base pairs. Additionally, approximately 3,000 SNPs causing amino acid substitutions in genes described in the literature as candidates for various diseases were used. The case-control study samples were of female Caucasian origin (British paternal and maternal descent) 670 individuals were equally distributed in two groups: female controls and female cases. The whole genome association approach was first conducted on 2 DNA pools representing the 2 groups. Significant markers were confirmed by individual genotyping.

TABLE 2

<u>General Statistics</u>		<u>Spacing Statistics</u>	
Total # of SNPs	25,488	Median	37,058 bp
# of Exonic SNPs	>4,335 (17%)	Minimum*	1,000 bp
# SNPs with refSNP ID	20,776 (81%)	Maximum*	3,000,000 bp
Gene Coverage	>10,000	Mean	122,412 bp
Chromosome Coverage	All	Std Deviation	373,325 bp
		<i>*Excludes outliers</i>	

Allelotyping and Genotyping Results

[0208] The genetic studies summarized above and described in more detail below identified allelic variants in the target genes that are associated with osteoarthritis.

Assay for Verifying, Allelotyping, and Genotyping SNPs

[0209] A MassARRAY™ system (Sequenom, Inc.) was utilized to perform SNP genotyping in a high-throughput fashion. This genotyping platform was complemented by a homogeneous, single-tube assay method (hME™ or homogeneous MassEXTEND™ (Sequenom, Inc.)) in which two genotyping primers anneal to and amplify a genomic target surrounding a polymorphic site of interest. A third primer (the MassEXTEND™ primer), which is complementary to the amplified target up to but not including the polymorphism, was then enzymatically extended one or a few bases through the polymorphic site and then terminated.

[0210] For each polymorphism, SpectroDESIGNER™ software (Sequenom, Inc.) was used to generate a set of PCR primers and a MassEXTEND™ primer which were used to genotype the polymorphism. Other primer design software could be used or one of ordinary skill in the art could manually design primers based on his or her knowledge of the relevant factors and considerations in designing such primers. Table 3 shows PCR primers and Table 4 shows extension primers used for analyzing polymorphisms. The initial PCR amplification reaction was performed in a 5 µl total volume containing 1X PCR buffer with 1.5 mM MgCl₂ (Qiagen), 200 µM each of dATP, dGTP, dCTP, dTTP (Gibco-BRL), 2.5 ng of genomic DNA, 0.1 units of HotStar DNA polymerase (Qiagen), and 200 nM each of forward and reverse PCR primers specific for the polymorphic region of interest.

TABLE 3: PCR Primers

SNP Reference	Forward PCR primer	Reverse PCR primer
rs910223	ACGTTGGATGACAGAGTGTCTCAGGGCTCAGA	ACGTTGGATGTGGTTTTCCAGTGTCTTAC
rs1367117	ACGTTGGATGTTGGTTTTCTTCAGCAAGGC	ACGTTGGATGAGCTTCATCCTGAAGACCAG
rs1024791	ACGTTGGATGGTGTAAAGGACTGCAGATAC	ACGTTGGATGAAACAGAACCCAGGAGGTTGG
rs1465621	ACGTTGGATGTTCTCCTCCCATTCTTCCTG	ACGTTGGATGGCGGGACTAGAAAGTAGATTCT
rs1018810	ACGTTGGATGTGCTGCTCCCATTCTCATG	ACGTTGGATGAAGGAGTAGAGACCTTGCTG
rs242392	ACGTTGGATGTGTTGGGCTGCTGTGGCTCT	ACGTTGGATGACCACTTCTCACGGTTACTG
rs8818	ACGTTGGATGAATCTCTCCCCTTCCAAAGC	ACGTTGGATGTCCCTGTGGTTTTTCATCCAC
rs1395486	ACGTTGGATGCTCATTATTTTCATGTTTAC	ACGTTGGATGTGCTGGAATAATGATTGTTG
rs512294	ACGTTGGATGTCTTGCTACCCACCTCCGAG	ACGTTGGATGAGAGCTCATGAGGGAATGGG

[0211] Samples were incubated at 95°C for 15 minutes, followed by 45 cycles of 95°C for 20 seconds, 56°C for 30 seconds, and 72°C for 1 minute, finishing with a 3 minute final extension at 72°C.

Following amplification, shrimp alkaline phosphatase (SAP) (0.3 units in a 2 µl volume) (Amersham Pharmacia) was added to each reaction (total reaction volume was 7 µl) to remove any residual dNTPs that were not consumed in the PCR step. Samples were incubated for 20 minutes at 37°C, followed by 5 minutes at 85°C to denature the SAP.

[0212] Once the SAP reaction was complete, a primer extension reaction was initiated by adding a polymorphism-specific MassEXTEND™ primer cocktail to each sample. Each MassEXTEND™ cocktail included a specific combination of dideoxynucleotides (ddNTPs) and deoxynucleotides (dNTPs) used to distinguish polymorphic alleles from one another. Methods for verifying, allelotyping and genotyping SNPs are disclosed, for example, in U.S. Pat. No. 6,258,538, the content of which is hereby incorporated by reference. In Table 4, ddNTPs are shown and the fourth nucleotide not shown is the dNTP.

TABLE 4: Extension Primers

SNP Reference	Extend Probe	Termination Mix
rs910223	GGGTCTGCACTGGTCCCA	ACT
rs1367117	AGCCATACACCTCTTTCAGG	ACT
rs1024791	CTGGCTGATGTCAGAAAGCA	ACG
rs1465621	CCATTCTTCCTGACATTTCGCC	CGT
rs1018810	CTGCTTTTATACATGCCACAC	ACT
rs242392	CTGCTGTGGCTCTACTGGT	ACG
rs8818	AGCCCCCAACCCACAGGCA	ACT
rs1395486	TTTCATGTTCAAAAAATCTTCT	ACG
rs512294	AGCTGGAGAGCAAACCACC	ACT

[0213] The MassEXTEND™ reaction was performed in a total volume of 9 µl, with the addition of 1X ThermoSequenase buffer, 0.576 units of ThermoSequenase (Amersham Pharmacia), 600 nM MassEXTEND™ primer, 2 mM of ddATP and/or ddCTP and/or ddGTP and/or ddTTP, and 2 mM of dATP or dCTP or dGTP or dTTP. The deoxy nucleotide (dNTP) used in the assay normally was complementary to the nucleotide at the polymorphic site in the amplicon. Samples were incubated at 94°C for 2 minutes, followed by 55 cycles of 5 seconds at 94°C, 5 seconds at 52°C, and 5 seconds at 72°C.

[0214] Following incubation, samples were desalted by adding 16 µl of water (total reaction volume was 25 µl), 3 mg of SpectroCLEAN™ sample cleaning beads (Sequenom, Inc.) and allowed to incubate for 3 minutes with rotation. Samples were then robotically dispensed using a piezoelectric dispensing device (SpectroJET™ (Sequenom, Inc.)) onto either 96-spot or 384-spot silicon chips containing a matrix

that crystallized each sample (SpectroCHIP™ (Sequenom, Inc.)). Subsequently, MALDI-TOF mass spectrometry (Biflex and Autoflex MALDI-TOF mass spectrometers (Bruker Daltonics) can be used) and SpectroTYPER RT™ software (Sequenom, Inc.) were used to analyze and interpret the SNP genotype for each sample.

Genetic Analysis

[0215] Minor allelic frequencies for the polymorphisms set forth in Table A were verified as being 10% or greater using the extension assay described above in a group of samples isolated from 92 individuals originating from the state of Utah in the United States, Venezuela and France (Coriell cell repositories).

[0216] Genotyping results are shown for female pools in Table 5. In Table 5, “AF” refers to allelic frequency; and “F case” and “F control” refer to female case and female control groups, respectively.

TABLE 5: Genotyping Results

SNP Reference	AF F case	AF F control	p-value
rs910223	A = 0.148 G = 0.852	A = 0.099 G = 0.901	0.0069
rs1367117	A = 0.339 G = 0.661	A = 0.402 G = 0.598	0.0181
rs1024791	G = 0.129 A = 0.871	G = 0.088 A = 0.912	0.0158
rs1465621	T = 0.071 A = 0.929	T = 0.107 A = 0.893	0.0194
rs1018810	A = 0.142 G = 0.858	A = 0.094 G = 0.906	0.0063
rs242392	C = 0.100 T = 0.900	C = 0.139 T = 0.861	0.0272
rs8818	G = 0.158 C = 0.842	G = 0.213 C = 0.787	0.0105
rs1395486	C = 0.115 T = 0.885	C = 0.158 T = 0.842	0.0231
rs512294	A = 0.078 G = 0.922	A = 0.124 G = 0.876	0.0054

[0217] All of the single marker alleles set forth in Table A were considered validated, since the genotyping data agreed with the allelotyping data and each SNP significantly associated with osteoarthritis. Particularly significant associations with osteoarthritis are indicated by a calculated p-value of less than 0.05 for genotype results.

Example 3

Association of Polymorphic Variants with Osteoarthritis in Replication Cohorts

[0218] The single marker polymorphisms set forth in Table A were genotyped again in two replication cohorts consisting of individuals selected for OA.

Sample Selection and Pooling Strategies – Replication Sample 1

[0219] A second case control sample (replication sample #1) was created by using 100 Caucasian female cases from Chingford, UK, and 148 unrelated female cases from the St. Thomas twin study. Cases were defined as having Kellgren-Lawrence (KL) scores of at least 2 in at least one knee x-ray. In addition, 199 male knee replacement cases from Nottingham were included. (For a cohort description, see the Nottingham description provided in Example 1). The control pool was made up of unrelated female individuals from the St. Thomas twin study (England) with normal knee x-rays and without other indications of OA, regardless of anatomical location, as well as lacking family history of OA. The St. Thomas twin study consists of Caucasian, female participants from the St. Thomas' Hospital, London, adult-twin registry, which is a voluntary registry of >4,000 twin pairs ranging from 18 to 76 years of age. The replication sample 1 cohort was used to replicate the initial results. Table 6 below summarizes the selected phenotype data collected from the case and control individuals.

TABLE 6

Phenotype	Female cases (n=248):	Male cases (n=199):	Female controls (n=313):
	median (range)/ (n,%)	median (range)/ (n,%)	mean (range)/ (n,%)
Age	59 (39- 73)	66 (45- 73)	55 (50- 72)
Height (cm)	162 (141- 178)	175 (152- 198)	162 (141- 176)
Weight (kg)	68 (51- 123)	86 (62- 127)	64 (40- 111)
Body mass index (kg/m ²)	26 (18- 44)	29 (21- 41)	24 (18- 46)
Kellgren-Lawrence* left knee	0 (63, 26%), 1 (20, 8%), 2 (105, 43%), 3 (58, 23%), 4 (1, 0%)	NA	NA
Kellgren-Lawrence* right knee	0 (43, 7%), 1 (18, 7%), 2 (127, 52%), 3 (57, 23%), 4 (1, 0%)	NA	NA
KL* >2 both knees	No (145, 59%), Yes (101, 41%)	NA	NA
KL* >2 either knee	No (0, 0%), Yes (248, 100%)	NA	NA

* 0: normal, 1: doubtful, 2: definite osteophyte (bony protuberance), 3: joint space narrowing (with or without osteophyte), 4: joint deformity

Sample Selection and Pooling Strategies – Replication Sample 2

[0220] A third case control sample (replication sample #2) was created by using individuals with symptoms of OA from Newfoundland, Canada. These individuals were recruited and examined by rheumatologists. Affected joints were x-rayed and a final diagnosis of definite or probable OA was made according to American College of Rheumatology criteria by a single rheumatologist to avoid any inter-examiner diagnosis variability. Controls were recruited from volunteers without any symptoms from the musculoskeletal system based on a normal joint exam performed by a rheumatologist. Only cases with a diagnosis of definite OA were included in the study. Only individuals of Caucasian origin were included. The cases consisted of 228 individuals with definite knee OA, 106 individuals with definite hip OA, and 74 individuals with hip OA.

TABLE 7

Phenotype	Case	Control
Age at Visit	62.7	52.5
Sex (Female/Male)	227/119	174/101
Knee OA Xray: No	35% (120)	80% (16)
Unknown	1% (4)	0% (0)
Yes	64% (221)	20% (4)
Hip OA Xray: No	63% (215)	80% (16)
Unknown	2% (7)	0% (0)
Yes	35% (121)	20% (4)

Assay for Verifying, Allelotyping, and Genotyping SNPs

[0221] Genotyping of the replication cohorts described in Tables 6 and 7 was performed using the same methods used for the original genotyping, as described herein. A MassARRAY™ system (Sequenom, Inc.) was utilized to perform SNP genotyping in a high-throughput fashion. This genotyping platform was complemented by a homogeneous, single-tube assay method (hME™ or homogeneous MassEXTEND™ (Sequenom, Inc.)) in which two genotyping primers anneal to and amplify a genomic target surrounding a polymorphic site of interest. A third primer (the MassEXTEND™ primer), which is complementary to the amplified target up to but not including the polymorphism, was then enzymatically extended one or a few bases through the polymorphic site and then terminated.

[0222] For each polymorphism, SpectroDESIGNER™ software (Sequenom, Inc.) was used to generate a set of PCR primers and a MassEXTEND™ primer which were used to genotype the polymorphism. Other primer design software could be used or one of ordinary skill in the art could manually design primers based on his or her knowledge of the relevant factors and considerations in designing such primers. Table 3 shows PCR primers and Table 4 shows extension probes used for analyzing (*e.g.*, genotyping) polymorphisms in the replication cohorts. The initial PCR amplification reaction was performed in a 5 µl total volume containing 1X PCR buffer with 1.5 mM MgCl₂ (Qiagen), 200 µM each of dATP, dGTP, dCTP, dTTP (Gibco-BRL), 2.5 ng of genomic DNA, 0.1 units of HotStar DNA polymerase (Qiagen), and 200 nM each of forward and reverse PCR primers specific for the polymorphic region of interest.

[0223] Samples were incubated at 95°C for 15 minutes, followed by 45 cycles of 95°C for 20 seconds, 56°C for 30 seconds, and 72°C for 1 minute, finishing with a 3 minute final extension at 72°C. Following amplification, shrimp alkaline phosphatase (SAP) (0.3 units in a 2 µl volume) (Amersham Pharmacia) was added to each reaction (total reaction volume was 7 µl) to remove any residual dNTPs that were not consumed in the PCR step. Samples were incubated for 20 minutes at 37°C, followed by 5 minutes at 85°C to denature the SAP.

[0224] Once the SAP reaction was complete, a primer extension reaction was initiated by adding a polymorphism-specific MassEXTEND™ primer cocktail to each sample. Each MassEXTEND™ cocktail included a specific combination of dideoxynucleotides (ddNTPs) and deoxynucleotides (dNTPs) used to distinguish polymorphic alleles from one another. Methods for verifying, allelotyping and genotyping SNPs are disclosed, for example, in U.S. Pat. No. 6,258,538, the content of which is hereby incorporated by reference. In Table 7, ddNTPs are shown and the fourth nucleotide not shown is the dNTP.

[0225] The MassEXTEND™ reaction was performed in a total volume of 9 µl, with the addition of 1X ThermoSequenase buffer, 0.576 units of ThermoSequenase (Amersham Pharmacia), 600 nM MassEXTEND™ primer, 2 mM of ddATP and/or ddCTP and/or ddGTP and/or ddTTP, and 2 mM of dATP or dCTP or dGTP or dTTP. The deoxy nucleotide (dNTP) used in the assay normally was complementary to the nucleotide at the polymorphic site in the amplicon. Samples were incubated at 94°C for 2 minutes, followed by 55 cycles of 5 seconds at 94°C, 5 seconds at 52°C, and 5 seconds at 72°C.

[0226] Following incubation, samples were desalted by adding 16 µl of water (total reaction volume was 25 µl), 3 mg of SpectroCLEAN™ sample cleaning beads (Sequenom, Inc.) and allowed to incubate for 3 minutes with rotation. Samples were then robotically dispensed using a piezoelectric dispensing

device (SpectroJET™ (Sequenom, Inc.)) onto either 96-spot or 384-spot silicon chips containing a matrix that crystallized each sample (SpectroCHIP™ (Sequenom, Inc.)). Subsequently, MALDI-TOF mass spectrometry (Biflex and Autoflex MALDI-TOF mass spectrometers (Bruker Daltonics) can be used) and SpectroTYPER RT™ software (Sequenom, Inc.) were used to analyze and interpret the SNP genotype for each sample.

Genetic Analysis

[0227] Genotyping results for replication cohorts #1 and #2 are provided in Tables 8 and 9, respectively.

TABLE 8

rsID	Replication #1 (Mixed Male/Female cases and Female controls)				Meta-analysis Disc. + Rep #1
	AF OA Con	AF OA Cas	Delta	P-value	P-value
rs910223	0.87	0.86	0.01	0.650	0.1800
rs1367117	0.67	0.64	0.03	0.182	0.9900
rs1024791	0.87	0.87	-0.01	0.718	0.5900
rs1465621	0.89	0.91	-0.02	0.209	0.0095
rs1018810	0.91	0.89	0.02	0.289	0.0062
rs242392	0.87	0.87	0.00	0.927	0.2400
rs8818	0.78	0.81	-0.03	0.259	0.0150
rs1395486	0.87	0.88	-0.01	0.492	0.0390
rs512294	0.89	0.88	0.00	0.909	0.3600

TABLE 9

rsID	Replication #2 (Newfoundland) (Male/Female cases and controls)				Meta-analysis Disc. + Rep #2
	AF OA Con	AF OA Cas	Delta	P-value	Not Done
rs910223	0.86	0.86	0.001	0.974	
rs1367117	0.64	0.69	-0.049	0.081	
rs1024791	0.87	0.87	0.006	0.767	
rs1465621	0.92	0.92	0.003	0.837	
rs1018810					
rs242392	0.88	0.88	-0.005	0.813	
rs8818	0.85	0.82	0.034	0.127	
rs1395486	0.86	0.85	0.015	0.486	
rs512294	0.90	0.93	-0.037	0.021	

[0228] To combine the evidence for association from multiple sample collections, a meta-analysis procedure was employed. The allele frequencies were compared between cases and controls within the discovery sample, as well as within the replication cohort #1 using the DerSimonian-Laird approach (DerSimonian, R. and N. Laird. 1986. Meta-analysis in clinical trials. Control Clin Trials 7: 177-188.)

[0229] The absence of a statistically significant association in one or more of the replication cohorts should not be interpreted as minimizing the value of the original finding. There are many reasons why a biologically derived association identified in a sample from one population would not replicate in a sample from another population. The most important reason is differences in population history. Due to bottlenecks and founder effects, there may be common disease predisposing alleles present in one population that are relatively rare in another, leading to a lack of association in the candidate region. Also, because common diseases such as arthritis-related disorders are the result of susceptibilities in many genes and many environmental risk factors, differences in population-specific genetic and environmental backgrounds could mask the effects of a biologically relevant allele. For these and other reasons, statistically strong results in the original, discovery sample that did not replicate in one or more of the replication samples may be further evaluated in additional replication cohorts and experimental systems.

[0230] *APOB*, *IL1RL2*, *WASPIP*, *BVES*, *LOXL1* and *CASPR4* regions were analyzed further, as shown in the examples below. *PADI2*, described above, is a peptidyl arginine deiminase enzyme, type II, that converts arginine residues within proteins to citrulline residues. This gene is one of four known *PADI* genes that encode enzymes that catalyze conversion of arginine to citrulline in proteins. Individuals with rheumatoid arthritis (RA) frequently have autoantibodies to citrullinated peptides, suggesting the involvement of the peptidylarginine deiminases citrullinating enzymes in RA (van Venrooij et al., *Arthritis Res.*;2(4):249-51. Epub 2000 May 24).

[0231] Pellino homolog 2 from *Drosophila* (*PELI2*) is a member of the Pellino gene family, which are involved in Toll-like signalling pathways. Pellino-2 associates with the pelle-like kinase/IL-1R-associated kinase protein to couple the pelle-like kinase/IL-1R-associated kinase protein to IL-1- or LPS-dependent signaling. *PELI2* may act as a downstream effector of interleukin receptor signaling and may play a role in inflammation-mediated Osteoarthritis. Pathway members downstream of *PELI2* may be targetable (e.g., interleukin receptors).

[0232] G protein-coupled receptor 50 (*GPR50*) is a member of the G protein-coupled receptor family. *GPR50* has significant homology to melatonin receptors and was isolated by PCR of human genomic DNA with degenerate primers based on conserved regions of melatonin receptors.

Example 4

IL1RL2 Proximal SNPs

[0233] It has been discovered that rs1024791, which lies within the IL1RL2 gene, is associated with occurrence of osteoarthritis in subjects. Interleukin-1 receptor-like 2 is a member of the interleukin 1 receptor family. IL1RL2 inhibits IL-1 activity and contains immunoglobulin domains. An experiment with transient gene expression demonstrated that this receptor was incapable of binding to interleukin 1 alpha and interleukin 1 beta with high affinity. This gene and four other interleukin 1 receptor family genes, including interleukin 1 receptor, type I (IL1R1), interleukin 1 receptor, type II (IL1R2), interleukin 1 receptor-like 1 (IL1RL1), and interleukin 18 receptor 1 (IL18R1), form a cytokine receptor gene cluster in a region mapped to chromosome 2q12. IL1RL2 may mediate inflammatory responses that can contribute to the development of OA. IL1RL2 biological activity can be modulated by addition of an antibody, a recombinant binding partner, a binding agent, or a recombinant IL1RL2 protein or functional fragment thereof.

[0234] One hundred forty additional allelic variants proximal to rs1024791 were identified and subsequently allelotyped in osteoarthritis case and control sample sets as described in Examples 1 and 2. The polymorphic variants are set forth in Table 10. The chromosome positions provided in column four of Table 10 are based on Genome “Build 34” of NCBI’s GenBank.

TABLE 10

dbSNP rs#	Chromosome	Position in SEQ ID NO: 2	Chromosome Position	Allele Variants
rs3917304	2	225	102409525	G/T
rs2041747	2	509	102409809	C/T
rs3917305	2	860	102410160	C/T
rs3771200	2	874	102410174	C/T
rs3917306	2	939	102410239	A/G
rs3917307	2	1483	102410783	G/T
rs3917308	2	1798	102411098	C/T
rs3917310	2	2189	102411489	A/T
rs3917311	2	2215	102411515	A/G
rs3917312	2	2282	102411582	C/G
rs3917313	2	2340	102411640	C/T
rs3917314	2	2963	102412263	A/C
rs3917316	2	3369	102412669	-/T
rs3171845	2	3481	102412781	A/G
rs3171846	2	3564	102412864	G/T
rs3917317	2	3653	102412953	-/TC
rs3917318	2	4860	102414160	A/G
rs3917319	2	4941	102414241	A/T

dbSNP rs#	Chromosome	Position in SEQ ID NO: 2	Chromosome Position	Allele Variants
rs3917320	2	4975	102414275	A/C
rs3917321	2	5321	102414621	A/G
rs3917322	2	5346	102414646	A/G
rs3917323	2	5541	102414841	A/G
rs3917324	2	5633	102414933	C/G
rs3917325	2	6007	102415307	G/T
rs3732134	2	6317	102415617	C/G
rs3732133	2	6378	102415678	A/G
rs2110726	2	6382	102415682	C/T
rs3917326	2	6426	102415726	C/T
rs3917327	2	6479	102415779	C/G
rs3917328	2	6641	102415941	C/T
rs3732131	2	6703	102416003	C/T
rs3732130	2	6705	102416005	C/T
rs3917329	2	7963	102417263	G/T
rs3917330	2	8525	102417825	G/T
rs3917331	2	8526	102417826	A/T
rs3917344	2	8598	102417898	C/T
rs3917332	2	8624	102417924	A/T
rs3917333	2	8883	102418183	A/T
rs3917334	2	8980	102418280	G/T
rs1030021	2	13578	102422878	G/T
rs2241132	2	16135	102425435	G/T
rs2241131	2	16141	102425441	G/T
rs3835036	2	16642	102425942	-/TGG
rs1997504	2	16931	102426231	A/G
rs1805232	2	17004	102426304	A/G
rs1971696	2	17009	102426309	C/T
rs1971695	2	17010	102426310	A/G
rs3771199	2	18713	102428013	C/T
rs1922303	2	18853	102428153	C/T
rs3213734	2	20783	102430083	C/T
rs1997503	2	21335	102430635	A/G
rs1558649	2	22180	102431480	C/T
rs1558648	2	22268	102431568	A/C
rs1558647	2	22285	102431585	C/T
rs1558646	2	25378	102434678	C/T
rs1882514	2	25906	102435206	C/G
rs1882513	2	26015	102435315	A/G
rs867770	2	26475	102435775	A/G
rs2310235	2	26798	102436098	A/T
rs870684	2	27042	102436342	A/G
rs3771197	2	27649	102436949	A/G
rs3771196	2	27827	102437127	A/T
rs3821207	2	27873	102437173	A/G
rs3771195	2	28122	102437422	A/G

dbSNP rs#	Chromosome	Position in SEQ ID NO: 2	Chromosome Position	Allele Variants
rs3771194	2	28202	102437502	A/G
rs3771193	2	28232	102437532	A/C
rs3771192	2	28240	102437540	G/T
rs3755290	2	29546	102438846	G/T
rs3821206	2	29748	102439048	A/G
rs2302623	2	30054	102439354	A/T
rs3755289	2	30646	102439946	G/T
rs1922302	2	31149	102440449	A/C
rs2110725	2	36912	102446212	A/C
rs1465326	2	36936	102446236	C/G
rs2871458	2	37184	102446484	C/T
rs2080310	2	39064	102448364	C/T
rs1922289	2	39343	102448643	G/T
rs1922290	2	40868	102450168	C/G
rs1922291	2	40917	102450217	A/G
rs1922292	2	41113	102450413	A/C
rs3815517	2	47343	102456643	A/T
rs2241130	2	47806	102457106	A/G
rs1922295	2	47911	102457211	A/G
rs1922294	2	48009	102457309	C/T
rs2302622	2	48621	102457921	C/G
rs2310240	2	49245	102458545	C/G
rs1024792	2	49247	102458547	C/G
rs3836112	2	49299	102458599	-/CTCT
rs3074969	2	49302	102458602	-/AGAG
rs917994	2	49514	102458814	C/T
rs2041753	2	49626	102458926	G/T
rs2041752	2	49791	102459091	A/G
rs1024791	2	50010	102459310	A/G
rs1024790	2	50294	102459594	A/G
rs995515	2	51482	102460782	A/G/T
rs995514	2	51556	102460856	A/G
rs1922293	2	51855	102461155	A/G
rs3755287	2	51956	102461256	C/T
rs3729564	2	52155	102461455	A/G
rs3771188	2	52448	102461748	A/G
rs3771187	2	52458	102461758	C/T
rs3771186	2	52511	102461811	C/T
rs3771185	2	52607	102461907	A/G
rs2310241	2	54049	102463349	A/C
rs2302621	2	54224	102463524	A/C
rs2302620	2	54567	102463867	A/G
rs3771184	2	55052	102464352	C/T
rs3834161	2	55857	102465157	-/C
rs3755286	2	55941	102465241	C/G
rs3755285	2	56120	102465420	A/G

dbSNP rs#	Chromosome	Position in SEQ ID NO: 2	Chromosome Position	Allele Variants
rs1997502	2	56349	102465649	C/T
rs3771182	2	56727	102466027	A/G
rs3836111	2	57232	102466532	-/CT
rs3771181	2	58806	102468106	C/T
rs955754	2	61181	102470481	C/T
rs2302612	2	63808	102473108	A/G
rs3755284	2	64526	102473826	A/T
rs3821205	2	64865	102474165	A/G
rs3815511	2	64928	102474228	C/T
rs2287041	2	64966	102474266	A/C
rs2287040	2	65080	102474380	A/G
rs2287039	2	65690	102474990	C/T
rs3755283	2	66228	102475528	A/G
rs3755282	2	66982	102476282	A/G
rs1812326	2	72511	102481811	A/G
rs1558626	2	74170	102483470	A/T
rs1558625	2	74264	102483564	C/T
rs1558624	2	74333	102483633	C/T
rs1558623	2	74502	102483802	A/T
rs1035131	2	74741	102484041	A/C
rs2110661	2	75321	102484621	C/T
rs1420093	2	82558	102491858	A/G
rs3074971	2	85366	102494666	-/TTG
rs1345302	2	85469	102494769	C/T
rs1420092	2	86485	102495785	G/T
rs1345301	2	87687	102496987	C/T
rs2310242	2	89463	102498763	G/T
rs2310243	2	89660	102498960	A/G
rs1882510	2	95718	102505018	C/T
rs1882511	2	95821	102505121	A/G

Assay for Verifying and Allelotyping SNPs

[0235] The methods used to verify and allelotype the 140 proximal SNPs of Table 10 are the same methods described in Examples 1 and 2 herein. The primers and probes used in these assays are provided in Table 11 and Table 12, respectively.

TABLE 11

dbSNP rs#	Forward PCR primer	Reverse PCR primer
rs3917304	ACGTTGGATGCAGAGAAGATAAGGAATGAG	ACGTTGGATGAAGGAAAATTACCCTAAACC
rs2041747	ACGTTGGATGGGGAAGACTATTACAGGTATG	ACGTTGGATGTAGGAGCAACTAACACTTGC

dbSNP rs#	Forward PCR primer	Reverse PCR primer
rs3917305	ACGTTGGATGGTTGTGAAGGAGAGGTCATG	ACGTTGGATGCGAAAGCCTCTACTGGTTTC
rs3771200	ACGTTGGATGCTGGTTTCCTACTGCTCATC	ACGTTGGATGAGTGCTTTGCAGGTGTTGTG
rs3917306	ACGTTGGATGCACCTGCAAAGCACTTTGTC	ACGTTGGATGTGCATTGTGTTCTCCATGGG
rs3917307	ACGTTGGATGCTGTAGTAAGATTCCATGAC	ACGTTGGATGACCCAAGTAATGAGGAAGTG
rs3917308	ACGTTGGATGCAGTGACTTCTGATGTCCTC	ACGTTGGATGAAGTTAGGTCTGGTACATTG
rs3917310	ACGTTGGATGGAGAAGAACTAAATGGAAGG	ACGTTGGATGGGGAAGAACTGATATCTTCA
rs3917311	ACGTTGGATGCCATAGATTCAATTTGGGGAAG	ACGTTGGATGGAGAAGAACTAAATGGAAGG
rs3917312	ACGTTGGATGCCATACAAACACTGACTCTC	ACGTTGGATGGAAGATATCAGTTCTTCCCC
rs3917313	ACGTTGGATGCACCATGACTATACTTGGTC	ACGTTGGATGTCAGTGTTTGTATGGGTGTG
rs3917314	ACGTTGGATGGGCCTGCATTGAGACAATAT	ACGTTGGATGGAACTTCATAGAATGCACC
rs3917316	ACGTTGGATGAGTATTCTTGTATATGCCAC	ACGTTGGATGGTTAGGAGATGTAGAAGATG
rs3171845	ACGTTGGATGGAAGCTATTAGGCTGAATATC	ACGTTGGATGACAGATGCTCTAAATACCTG
rs3171846	ACGTTGGATGTGTCCTATTATCATCACAGAGC	ACGTTGGATGCTGCCTCAACATTCATATTGG
rs3917317	ACGTTGGATGTCTCAGCCCTGAATTCATATC	ACGTTGGATGGACTAGATCTTCATGCATCAG
rs3917318	ACGTTGGATGAAAAGCCTTGTGTGGCTTTG	ACGTTGGATGGTCTGAAAAACAGGAAGCAC
rs3917319	ACGTTGGATGGTGCTTCCTGTTTTTCAGAC	ACGTTGGATGAAGCCTGATGTTTCTCTGAC
rs3917320	ACGTTGGATGCGTAAAGAAAAGCAGAAGAC	ACGTTGGATGTTGCTCTTCAGATGAACCAC
rs3917321	ACGTTGGATGAGGAGAACTGCAAAGAGAG	ACGTTGGATGACAGGAGGCACCTAAAGAAC
rs3917322	ACGTTGGATGAGTCAGCATGAGGCATAACC	ACGTTGGATGAGCATGGAGAAGTTGCCAAG
rs3917323	ACGTTGGATGACTTCAGAGTAGAGGGCTTG	ACGTTGGATGAAGTGCTGGGATTATAGGCG
rs3917324	ACGTTGGATGATCACCAGAGGTCAGGAGTT	ACGTTGGATGCCACCATGCCTAGCTCATTT
rs3917325	ACGTTGGATGTAGTTAAGTCATCCACAGCC	ACGTTGGATGTGTCAGTCTCACTTTGCCTG
rs3732134	ACGTTGGATGTTAATGCTTTCCTCCCTGGC	ACGTTGGATGTAGGGAGCTGTTCTCTCCAAA
rs3732133	ACGTTGGATGAAGGATGGTTCATGTGTGGG	ACGTTGGATGTTACGTCTTTGGAGGAACAG
rs2110726	ACGTTGGATGAAGGATGGTTCATGTGTGGG	ACGTTGGATGTACGTCTTTGGAGGAACAGC
rs3917326	ACGTTGGATGTGCACAGCCACACATGAAC	ACGTTGGATGTTGAGCTCCTGAACAGGTGG
rs3917327	ACGTTGGATGAAAGCATGGGCTTCAGCTCC	ACGTTGGATGATGCCGCTCTTCTGTCTATCC
rs3917328	ACGTTGGATGTAGGCAAAGGAGGAGGAAGG	ACGTTGGATGTGTGTGAATCCCAGGTTGG
rs3732131	ACGTTGGATGAGGCCTTCTCGCATTTTCTC	ACGTTGGATGTCCCAGAGACTGTGGAATTG
rs3732130	ACGTTGGATGTCCCAGAGACTGTGGAATTG	ACGTTGGATGAGGCCTTCTCGCATTTTCTC
rs3917329	ACGTTGGATGAAGTCAAAGGAAGTTCACGG	ACGTTGGATGGTGCAAAGTTATCCCCATC
rs3917330	ACGTTGGATGTAAGCCAATAGCCTCTGACC	ACGTTGGATGAACAAGGTGAGGAGACCTTC
rs3917331	ACGTTGGATGAACAAGGTGAGGAGACCTTC	ACGTTGGATGTAAGCCAATAGCCTCTGACC
rs3917344	ACGTTGGATGAGAGTTCTTCTGTTGTGGG	ACGTTGGATGTAGAAGAAGGGAGTTAGGGC
rs3917332	ACGTTGGATGATTGGCTTAACAGTGAGCCC	ACGTTGGATGAGAAGCAAATGAGCAGAGGG
rs3917333	ACGTTGGATGTAAGAAGGAGGCACTGACTG	ACGTTGGATGGCTGTCCAAAATGCATGCTC
rs3917334	ACGTTGGATGGACTCAGACTCTAAGCCAAC	ACGTTGGATGAGTCAGTGCCTCCTTCTTAC
rs1030021	ACGTTGGATGCATTGCTTCATGTTCTTACC	ACGTTGGATGAAAAGTGGGCATAACCTCTC
rs2241132	ACGTTGGATGAGGAGGATGGGCGAGGAGTA	ACGTTGGATGTCTGGACACCAGCCTGCTTC
rs2241131	ACGTTGGATGAGGAGGATGGGCGAGGAGTA	ACGTTGGATGCTGTCAGGTGGCAGAAGCAG
rs3835036	ACGTTGGATGTTCCGCGGAAGAGGAAACAG	ACGTTGGATGTCACCTCCAAGCTCAAAGGC
rs1997504	ACGTTGGATGCCTGTAAATCCCAGTACTTTG	ACGTTGGATGTGTTAGCCAGGATGGTCTAG
rs1805232	ACGTTGGATGTTGAGTAGCTGGGACTACAG	ACGTTGGATGTAACACGGTGAAACCCCGTC
rs1971696	ACGTTGGATGTAGACCATCCTGGCTAACAC	ACGTTGGATGTTGAGTAGCTGGGACTACAG
rs1971695	ACGTTGGATGTTGAGTAGCTGGGACTACAG	ACGTTGGATGTAGACCATCCTGGCTAACAC
rs3771199	ACGTTGGATGTGAATAACACAGGCCTGCTG	ACGTTGGATGGCTTGACCTGAATAGACAGC

dbSNP rs#	Forward PCR primer	Reverse PCR primer
rs1922303	ACGTTGGATGGTGGGGCCTGAATAAAACAC	ACGTTGGATGTAAGGTCATGCAAGCCAGTG
rs3213734	ACGTTGGATGAACCCACTGTTTTTATAGG	ACGTTGGATGTGACTGCTAGCTAACTAATC
rs1997503	ACGTTGGATGAAAACTCATGACCCAGAGGG	ACGTTGGATGGCACAGGCTAGTCATTTGAG
rs1558649	ACGTTGGATGTGCATGGTGGTTCATGCCTG	ACGTTGGATGAATCTTGCTATGATGCCAG
rs1558648	ACGTTGGATGAGATTTCTACAACCTTG TG	ACGTTGGATGAGGTACATTTTATACCCACC
rs1558647	ACGTTGGATGGAAAAATGTGGTCAATCTCAC	ACGTTGGATGCAACCTTGTTGTTGAACCTTG
rs1558646	ACGTTGGATGGGCCCTTGTTAGAGTTTAGG	ACGTTGGATGGCTTTAGGTTGGCATAAATGG
rs1882514	ACGTTGGATGTTCTTTCTGTCCATCCTG	ACGTTGGATGCAGAGTTGAGGTACTGGAAG
rs1882513	ACGTTGGATGAAAGTAGAGAGGTCAGGTGG	ACGTTGGATGGGGCATTACACTTTTCCACC
rs867770	ACGTTGGATGGCAGGTGGTGTATTTAGAG	ACGTTGGATGACACTGCAGAAGTAGCTTGC
rs2310235	ACGTTGGATGGAGCTGGAATAGGGAATCAG	ACGTTGGATGGCCATTATCCAGAACCTCTG
rs870684	ACGTTGGATGCCCAAATTACTCCTCAGCAC	ACGTTGGATGAGAGCGCGAAGTAACCTCAG
rs3771197	ACGTTGGATGTAAGCAGTTCAGTCCACAG	ACGTTGGATGCCTTTGCTTACCTAAGACTG
rs3771196	ACGTTGGATGCCTTTAACTACACAGCAAC	ACGTTGGATGAGAAGCTTTCTGAGCAAGAG
rs3821207	ACGTTGGATGAAAACCATGAAGAGGAGACG	ACGTTGGATGGCAACTAAAGGATCTTCTC
rs3771195	ACGTTGGATGGTGGACGCTATTGTTCTTAAC	ACGTTGGATGTAACTCTCAATGAGCTTGG
rs3771194	ACGTTGGATGATCTTAAAGTTCAGCCTTGC	ACGTTGGATGATAATGTTCCAGTGGATCAG
rs3771193	ACGTTGGATGGTTCAGTGGATCAGAATAG	ACGTTGGATGTTAAAGTTCAGCCTTGCAGC
rs3771192	ACGTTGGATGGGGTTCATTCTTTCTTTCAAG	ACGTTGGATGATAGCAAAGCGACAGAATGG
rs3755290	ACGTTGGATGCCCAATTACACTTTCTGCAC	ACGTTGGATGTGATCACTGTTCCAGACCTTC
rs3821206	ACGTTGGATGAGAGTGGCCTACATGAGTTG	ACGTTGGATGCCTCCTGCAAAAACTGACC
rs2302623	ACGTTGGATGGAATACTTAGAAACCTGTGTG	ACGTTGGATGATCTGTTGTCTTCCAGTTAG
rs3755289	ACGTTGGATGTCCAGAACTCTGAGCTCTGC	ACGTTGGATGCCTCAGCCTTCATTGTCGTG
rs1922302	ACGTTGGATGGAGATCTTTCACCTTCTTGG	ACGTTGGATGGCCACACATAAAACCATATC
rs2110725	ACGTTGGATGATTCTCTCCCAAGCTATAC	ACGTTGGATGCAATAACCAGGTTTGTGACC
rs1465326	ACGTTGGATGTGTGTTTGAAAAACCAATG	ACGTTGGATGTTTACAGAGTTCAGGAGGG
rs2871458	ACGTTGGATGAGATCCCCATAGGGATCCAC	ACGTTGGATGCACACTTCAGAGTACTAGGG
rs2080310	ACGTTGGATGGAATGATCCATTCCAGGGTG	ACGTTGGATGGACATCATGTTACCTGTGCC
rs1922289	ACGTTGGATGTGAGTTTGGTCATTGCTACG	ACGTTGGATGATACAGGCCATGACCTACTC
rs1922290	ACGTTGGATGACACCCAGTTTCCAGCTTTG	ACGTTGGATGCTTCGGCTCTCTGGTGTITT
rs1922291	ACGTTGGATGGACTTCTCTGCTACCACAAC	ACGTTGGATGCTCATGGGGAGAGGAATCAA
rs1922292	ACGTTGGATGATATTACCTCACAATGCAAG	ACGTTGGATGATGCTTATTGATCCTTTTCC
rs3815517	ACGTTGGATGACAATGGTTGTCCTGGAAGG	ACGTTGGATGAATAGCCCCCTAGGCAAATG
rs2241130	ACGTTGGATGGAGAAATGGATCTTACTGCTC	ACGTTGGATGCAATCCCACCTATCACATAG
rs1922295	ACGTTGGATGGTTATATCATGAGCCATCGG	ACGTTGGATGGTGTCAATTCAGTGTTTGC
rs1922294	ACGTTGGATGCGGGCATACAAAGCAAACAC	ACGTTGGATGACTGTCTTCCCTAAGAGTCC
rs2302622	ACGTTGGATGTACTCCAGTGGGTTACACAC	ACGTTGGATGGCATTAGAGTCACTGCTCC
rs2310240	ACGTTGGATGAAATTCAAGTCCTCTCTCTT	ACGTTGGATGGTGGTTTACCAAGACAGTTG
rs1024792	ACGTTGGATGGCTGTGTGGTTTACCAAGAC	ACGTTGGATGCCACACACGTGCGTGTCAA
rs3836112	ACGTTGGATGACGCACGTGTGTGGCTAGCTA	ACGTTGGATGGATGTATGCAAGCATAGG
rs3074969	ACGTTGGATGACGTGTGTGGCTAGCTACAT	ACGTTGGATGGGCTTTAGCTTGATGTATGC
rs917994	ACGTTGGATGCCTCCCTTAGAATTGCAGTG	ACGTTGGATGAAGCAGAGAATGTGCACACC
rs2041753	ACGTTGGATGTCCACATGTTGCAACCCAAG	ACGTTGGATGTAAGTGTGAGTGAGCACAGC
rs2041752	ACGTTGGATGGAACCTCTTAGAGGTACCAG	ACGTTGGATGTCCTTCTCCATCACTTTCCC
rs1024791	ACGTTGGATGGTGTAAAGGGACTGCAGATAC	ACGTTGGATGAAACAGAACCCAGGAGTTGG
rs1024790	ACGTTGGATGAGAAAAATCCAGCTGATTCT	ACGTTGGATGGACTCCTGCCCTACACTTTAA

dbSNP rs#	Forward PCR primer	Reverse PCR primer
rs995515	ACGTTGGATGTGGGATGGAAATCGCTATTG	ACGTTGGATGTGTCCCAACCTAGAAGTTTG
rs995514	ACGTTGGATGGCTTGGACTTGGCCTCAGAA	ACGTTGGATGCCAATAGCGATTTCCATCCC
rs1922293	ACGTTGGATGGGACAGAGCTAAGGTTATAG	ACGTTGGATGGATTCAAATCTGGAGGTGTC
rs3755287	ACGTTGGATGAAATTGGGTGTGCTCTTCCG	ACGTTGGATGGACTACTACCAGCCTTCAAC
rs3729564	ACGTTGGATGCCTGAGTCCCTCTGAATGTA	ACGTTGGATGTGCCTTCGAGAGTACTGATG
rs3771188	ACGTTGGATGAATCCAATCCTGGGCACTTG	ACGTTGGATGAGAGTAGAGGATGAGGAAGC
rs3771187	ACGTTGGATGAGAGTAGAGGATGAGGAAGC	ACGTTGGATGAATCCAATCCTGGGCACTTG
rs3771186	ACGTTGGATGAAGTGCCAGGATTGGATTG	ACGTTGGATGGAGTAAGTCCCAATGCAGCC
rs3771185	ACGTTGGATGATCTTGAGGCCCAAGATTTT	ACGTTGGATGGGCACCAAATGTGTTCTTAG
rs2310241	ACGTTGGATGACCTTCTCCAGCTGGTTCTG	ACGTTGGATGTGGGAGTCCAGCTGTTCAAC
rs2302621	ACGTTGGATGCGTCTACCACCGGAAACTAG	ACGTTGGATGGGAAACAAGTCAGCTCCTGG
rs2302620	ACGTTGGATGGTCTCTGTAGAATGGAAGGC	ACGTTGGATGTGGCTGTGTCTGTTGTGTAC
rs3771184	ACGTTGGATGTCTCTCTAGGCCCTGTACTT	ACGTTGGATGACTTGGTTTGATCTCTCTCC
rs3834161	ACGTTGGATGAGGGAACCTGGTTGTCTGAG	ACGTTGGATGCAAAGCAAGCACTTGATGCC
rs3755286	ACGTTGGATGGCATCAAGTGCTTGCTTTGC	ACGTTGGATGCAAGTTAGTGAATAGCCACG
rs3755285	ACGTTGGATGTGCAGATGCCAGAGCCAAAA	ACGTTGGATGACCTGAAGTGCTGCTAGTAC
rs1997502	ACGTTGGATGCGTATTCTTCTGGAAGCTC	ACGTTGGATGTCACTGACAGAGTCAGTGAG
rs3771182	ACGTTGGATGGCCAACACACAGAGATATTAC	ACGTTGGATGGTATGTGTGCATTTTGTGATG
rs3836111	ACGTTGGATGTCTACCCCGACTTGTTTTCC	ACGTTGGATGGGCTAAACGAAGACAAGCC
rs3771181	ACGTTGGATGTTCTTCTCCAAAAGTTCAG	ACGTTGGATGGCCAGAGGATTTTTTTTCCG
rs955754	ACGTTGGATGGTGATGTGGCCAGAAATGAG	ACGTTGGATGTATCCTCCTGCTTCAGCTTG
rs2302612	ACGTTGGATGTGACAAACCTCGTGTCTCTCC	ACGTTGGATGAAGGTGTCGGCCGTTTCTCTC
rs3755284	ACGTTGGATGGCTGCTCAGAATTCTGGTTG	ACGTTGGATGACCCCTCCATGTTTGAGAGG
rs3821205	ACGTTGGATGATGCCATCCTAAGACCACAG	ACGTTGGATGCTTAGTAAGCAGTCAGTGGG
rs3815511	ACGTTGGATGTACCACCCATCGCCTGTGAA	ACGTTGGATGGTGGTCTTAGGATGGCATGG
rs2287041	ACGTTGGATGTGAAAGTCCATCCACACTG	ACGTTGGATGTGTGGTCTTAGGATGGCATG
rs2287040	ACGTTGGATGATAAAGAGTGGACCAATGTC	ACGTTGGATGTTATGTTCCAAGGTGACCTC
rs2287039	ACGTTGGATGTTACAGGCACACCCTTCAG	ACGTTGGATGAGCCACAGTGTGGGGAGAGT
rs3755283	ACGTTGGATGTTCTTGCTGCATTGCATCCC	ACGTTGGATGGGAGAGAGAAATCGAGATGC
rs3755282	ACGTTGGATGGAGGACCAAGCAAGATGAAG	ACGTTGGATGATATTTTGGCAGGCCAGCTC
rs1812326	ACGTTGGATGTTCAAGTGATTCTACTGCCG	ACGTTGGATGAACCCCGTCTCTACTAAAC
rs1558626	ACGTTGGATGACCTCCAAGCATGATCTCAG	ACGTTGGATGTGGTTTTCCCTTGGTACTCG
rs1558625	ACGTTGGATGTCAGCAAAGCAGGACCGACC	ACGTTGGATGTGAGATCATGCTTGGAGGTC
rs1558624	ACGTTGGATGGGAAAGAACGGCCTGTCTTC	ACGTTGGATGATCCACAGGGTTCGTGTTGT
rs1558623	ACGTTGGATGAAGTCCCAAACCCAAGTGAG	ACGTTGGATGTTAGGAAGCGAAGGAAAAAC
rs1035131	ACGTTGGATGACTCTTCTACCTTGATGGC	ACGTTGGATGTAGGCTTCAGGATTGGATGG
rs2110661	ACGTTGGATGTCCCTCCAAAACCCACCTTT	ACGTTGGATGTGGATGGTGACACCTTCATG
rs1420093	ACGTTGGATGAAGAAATTTAAAGCCAGAG	ACGTTGGATGTATCTCAATAGAGGCTCTAC
rs3074971	ACGTTGGATGAAACAAACTGAACCGCTAGG	ACGTTGGATGCAGCGTCTCTGGGTATTT
rs1345302	ACGTTGGATGGGTAATCAGAAAACAGAGTC	ACGTTGGATGTGCCAGTAGAAGTACAGTAG
rs1420092	ACGTTGGATGGTGCTCAGAGATGGTTAAAC	ACGTTGGATGACTGCACCCTAGTTGATTTG
rs1345301	ACGTTGGATGGCTCAAGTCTGGAGAAATGA	ACGTTGGATGCATGGTTGGATTTTGTGTTG
rs2310242	ACGTTGGATGCCACCACTCAAACCTTTGTC	ACGTTGGATGGACAGCAAGAGTGAACTCC
rs2310243	ACGTTGGATGTGTAGCTAAGCACTATAGCG	ACGTTGGATGGCTCCTTCTAGATATGCATG
rs1882510	ACGTTGGATGCTCGCTAGTCACTGGAGCTG	ACGTTGGATGAAGTCCAGGTGGACCTGGT
rs1882511	ACGTTGGATGAAGGAAGTGTGAGGGCCATG	ACGTTGGATGAATGGTGCAACTGCCTTGGG

TABLE 12

dbSNP rs#	Extend Primer	Term Mix
rs3917304	GGTTACTAATGGTGGTTTTCTCTG	ACT
rs2041747	ATGCTAAGAGTTATTCACATTTTG	ACT
rs3917305	GGAGATCCTTGTCCCATAGAT	ACT
rs3771200	TACTGCTCATCTATGGGACAA	ACT
rs3917306	GCACTTTGTCATCTGCCCA	ACT
rs3917307	AAGTTTGAAATGCCATTTCTCT	ACT
rs3917308	TAGTCTTACCCTATGCATCATCA	ACT
rs3917310	ATGGAAGGATATACAATGTTTCAT	CGT
rs3917311	ATTCATTTGGGAAGAACTGATA	ACG
rs3917312	TACAAACACTGACTCTCACTTGTA	ACT
rs3917313	CTTGGTCCTTTACAGTTCCT	ACT
rs3917314	GGATACTAATGTACAAAGCAATGA	ACT
rs3917316	ATTTTAGAAACCCCTCTTAGTAAAA	CGT
rs3171845	TGAATATCATTGTTTTCTAA	ACT
rs3171846	ATCACAGAGCAAGGCCTA	CGT
rs3917317	AGTTTAAACAAAGGAGAGAGAGA	ACT
rs3917318	GTGTGGCTTTGGTTCAGGAG	ACT
rs3917319	GTTGAGGTCATTAATGAAACGT	CGT
rs3917320	GAAGACTGATTATCATTTTAGTC	CGT
rs3917321	ACGTGCCTCTCGGGTAGC	ACT
rs3917322	CCATAAGACAGGAGGCACC	ACG
rs3917323	GGGAAGATCTTTAAAAAGGCA	ACT
rs3917324	TCAGGAGTTCGAGACCAGC	ACT
rs3917325	CCTGTAGAGTCACTGACCC	ACT
rs3732134	TTCTCCCTGGCATGACCAT	ACT
rs3732133	CAAGGGACATTGCAGACGGA	ACT
rs2110726	TGCAAGGGACATTGCAGA	ACG
rs3917326	CCCACACATGAACCATCCTTCC	ACG
rs3917327	GCTTCAGCTCCTGAACAGGTG	ACT
rs3917328	GGAGGAAGGGTGCAGGCAA	ACT
rs3732131	TCTCGCATTTTCTCTAGCTGATC	ACT
rs3732130	GGATGTTCTGAATTTTGGTAAAAT	ACG
rs3917329	CTTCTTCCTCCAGAATTCAAC	CGT
rs3917330	TCCCCACAACAGGAAGAACT	CGT
rs3917331	TGAGGAGACCTTCTGCAGAG	CGT
rs3917344	GAGTGGAGGTCAGAGGCTAT	ACG
rs3917332	ACAGTGAGCCCTAACTCCC	CGT
rs3917333	GGGTGTCATCTCTGACCATC	CGT
rs3917334	TCAGACTCTAAGCCAACCTGCCA	CGT

dbSNP rs#	Extend Primer	Term Mix
rs1030021	CTTTTAAATTTTGCCAGTTTGC	ACT
rs2241132	CGGTGGGGACCGCGTGG	ACT
rs2241131	CGGCGGCGGTGGGGACC	ACT
rs3835036	GCGGAAGAGGAAACAGAGAACCA	ACT
rs1997504	GCGGGCGGATCACGAGG	ACG
rs1805232	CGCCCGCCACCGCGCCC	ACT
rs1971696	ACATTAAAAAATTAGCCGGGC	ACT
rs1971695	TACAGGCGCCCGCCACC	ACT
rs3771199	TGACTGTGGTCAGCTGGAAA	ACT
rs1922303	GGGGCCTGAATAAAACACATCTGT	ACT
rs3213734	TTTAAGGCAGAATTGGTAAAGAAA	ACT
rs1997503	AGAGGGGTGTGCTGGCAGGC	ACT
rs1558649	GGTGGTTCATGCCTGTAATCC	ACG
rs1558648	TGTTGAACCTTTGTATTATAAGCC	ACT
rs1558647	GGTACATTTTATACCCACCAAA	ACG
rs1558646	CTTGGTTAGAGTTTAGGGCACAT	ACT
rs1882514	GGATTCACGTGTCCATCACTT	ACT
rs1882513	GTGGGCTAATTCCAGTTAAGA	ACG
rs867770	AGAAGTAGCTTGCCCTGAGAGC	ACG
rs2310235	GGGAATCAGTCAGAAAGTAATA	CGT
rs870684	CACAGTGGTTTTGGGTCCC	ACG
rs3771197	GTTCCAGTCCACAGAATTTAGT	ACT
rs3771196	CTACACAGCAACTAAAGGATC	CGT
rs3821207	AAGAGGAGACGAGCATCAGA	ACT
rs3771195	TTAAATCTTGTTAGTGAGACATTA	ACG
rs3771194	TGTCGCTTTGCTATAACTTAGACT	ACT
rs3771193	GTTATAGCAAAGCGACAGAATG	ACT
rs3771192	CATCTTAAAGTTCAGCCTTGCA	ACT
rs3755290	TGCACTTATCAAGCATTGGAC	ACT
rs3821206	GGAAGGAAGACTTCATGGAG	ACT
rs2302623	GAAACCTGTGTGATCCCTAG	CGT
rs3755289	TCAGCTGGAAGGCCCGCA	ACT
rs1922302	TTAATTCCTAGGTATTTAATTTTCG	ACT
rs2110725	CATTTTACAGAGTTCCAGGAGGG	ACT
rs1465326	GGCTCTGTTTCTGACAATAACCAG	ACT
rs2871458	GGATCCACACCACCCAGAA	ACT
rs2080310	GGTGGATCAGAAAGTGCAGGT	ACT
rs1922289	CATTGCTACGTTGAGTATGAG	ACT
rs1922290	CCCAGTTTCCAGCTTTGGATATAC	ACT
rs1922291	TCTGCTACCACAACCTTTTCCA	ACG
rs1922292	ACCTCACAATGCAAGATATATTA	CGT
rs3815517	GCCACTTGCCCTTGTGG	CGT
rs2241130	GATCTTACTGCTCTCAGGGAT	ACT

dbSNP rs#	Extend Primer	Term Mix
rs1922295	GCCTTCAAAGCTTAATGCCC	ACG
rs1922294	GTTCTTTGCTATACTAAACAAGC	ACT
rs2302622	CACACTGTTGAGAGTGTTCAAAAC	ACT
rs2310240	TGCAAACACACACACACACACA	ACT
rs1024792	CGTGTCAAACACACACACACACA	ACT
rs3836112	TGGCTAGCTACATGCAAGAG	ACT
rs3074969	TGGCTAGCTACATGCAAGAG	ACT
rs917994	CAGTGAATAGGGATCTGTGC	ACT
rs2041753	CCCATGTGCTCAGGGTGAG	ACT
rs2041752	CTTAGAGGTACCAGAGAGAGA	ACT
rs1024791	CTGGCTGATGTCAGAAAGCA	ACG
rs1024790	CACAGAGAGGTTGAGTGACA	ACT
rs995515	CTATTGGTCAGCTTCAGTCTAT	ACT
rs995514	ACTTGGCCTCAGAATCCTTC	ACT
rs1922293	GCTTCTCCATTTGACTTCCTTA	ACG
rs3755287	GGTGTGCTCTTCCGTGAATTCGC	ACT
rs3729564	TTCCAATTTCACTCTCTTTAGCT	ACG
rs3771188	TGTGAGAACCCCTCACTTCA	ACT
rs3771187	TCTGTCTTATGATTGAAGTGAG	ACT
rs3771186	CGGTGTGTGGTGCACTGC	ACT
rs3771185	AGGCCCAAGATTTCTCATTTACT	ACT
rs2310241	CAGCTGGTTCTGCTGCCC	ACT
rs2302621	GGGCTCTGCAGACTTTTACTC	ACT
rs2302620	CTGTAGAATGGAAGGCACTTCG	ACT
rs3771184	CCCTGTACTTGGTGCTGAAG	ACT
rs3834161	GTTGTCTGAGAACGTTTATGGG	ACT
rs3755286	AGTACGGTTGTTGCCACAT	ACT
rs3755285	ACCCCTCCCATGCCC	ACT
rs1997502	TCCTGGAAGCTCAGGCCCC	ACT
rs3771182	GTTCTCGTAGACAGAGCTGT	ACT
rs3836111	CCTTGGTTTCCCTTTGATCACT	CGT
rs3771181	TCAGAAACATAAGAACTTATGAA	ACT
rs955754	GCCAGAAATGAGAATTAAAGGCAG	ACT
rs2302612	GTAGCAAGGTGTGTGCTGC	ACT
rs3755284	TGTCTAAAAGAGAGAGAAAAGG	CGT
rs3821205	CCTCTGGCTCCCTCTCTC	ACT
rs3815511	GGCACAGCACCTCCTAACC	ACG
rs2287041	CATCCACACTGGGTACCA	CGT
rs2287040	TGGACCAATGTCAAGTCGAG	ACT
rs2287039	CAGAGAGGACACGTCCCC	ACT
rs3755283	CCTATTATTTCACTAGGAATTAGT	ACT
rs3755282	CATGTGAAAAGTGCTTGGCAAAC	ACG
rs1812326	AGGTGCATGCCACCACACT	ACG

dbSNP rs#	Extend Primer	Term Mix
rs1558626	TTCAGGCTAGTTTCACCCGA	CGT
rs1558625	GCAGGACCGACCCTCCCT	ACG
rs1558624	GGCCTGTCTTCAGGGCTC	ACT
rs1558623	AAGTGAGGGCTCCAGCGAT	CGT
rs1035131	GATGGCACATCTCTAGAAAAG	CGT
rs2110661	GTCTCTCCTCAGATATGAGCC	ACG
rs1420093	TTTAAAGCCCAGAGATTTTAAAAA	ACT
rs3074971	CTAGGAAAAAAGAAAGGCAACA	CGT
rs1345302	GAAAACAGAGTCTTTACCAATC	ACT
rs1420092	AGAGATGGTTAAACAGGCACA	ACT
rs1345301	CACAAGTTTACACCTTTTCTTTA	ACT
rs2310242	CTCTATAACCTTACAAATGTTATT	CGT
rs2310243	TGCAGTTTGGGACACAAAGG	ACG
rs1882510	AAAAGTGAAGCTGGGCCTGC	ACT
rs1882511	GGGAGGCATTCAGGGATCA	ACG

Genetic Analysis

[0236] Allelotyping results from the discovery cohort are shown for cases and controls in Table 13. The allele frequency for the A2 allele is noted in the fifth and sixth columns for osteoarthritis case pools and control pools, respectively, where “AF” is allele frequency. The allele frequency for the A1 allele can be easily calculated by subtracting the A2 allele frequency from 1 (A1 AF = 1-A2 AF). For example, the SNP rs3917304 has the following case and control allele frequencies: case A1 (G) = 0.431; case A2 (T) = 0.569; control A1 (G) = 0.450; and control A2 (T) = 0.550, where the nucleotide is provided in paranthesis. Some SNPs are labeled “untyped” because of failed assays.

TABLE 13

dbSNP rs#	Position in SEQ ID NO: 2	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs3917304	225	102409525	G/T	0.569	0.550	0.460
rs2041747	509	102409809	C/T	0.027	0.023	0.800
rs3917305	860	102410160	C/T			
rs3771200	874	102410174	C/T	0.467	0.473	0.809
rs3917306	939	102410239	A/G			
rs3917307	1483	102410783	G/T			
rs3917308	1798	102411098	C/T			
rs3917310	2189	102411489	A/T			
rs3917311	2215	102411515	A/G	0.945	0.964	0.193
rs3917312	2282	102411582	C/G			
rs3917313	2340	102411640	C/T			
rs3917314	2963	102412263	A/C	0.025	0.028	0.881
rs3917316	3369	102412669	-/T	0.785	0.856	0.004
rs3171845	3481	102412781	A/G	0.904	0.894	0.624

dbSNP rs#	Position in SEQ ID NO: 2	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs3171846	3564	102412864	G/T			
rs3917317	3653	102412953	-/TC	0.320	0.325	0.824
rs3917318	4860	102414160	A/G	0.151	0.151	0.978
rs3917319	4941	102414241	A/T			
rs3917320	4975	102414275	A/C	0.936	0.946	0.585
rs3917321	5321	102414621	A/G			
rs3917322	5346	102414646	A/G	0.978	untyped	NA
rs3917323	5541	102414841	A/G	0.977	untyped	NA
rs3917324	5633	102414933	C/G			
rs3917325	6007	102415307	G/T	0.029	0.030	0.901
rs3732134	6317	102415617	C/G			
rs3732133	6378	102415678	A/G			
rs2110726	6382	102415682	C/T	0.320	0.318	0.944
rs3917326	6426	102415726	C/T			
rs3917327	6479	102415779	C/G			
rs3917328	6641	102415941	C/T	0.898	0.891	0.706
rs3732131	6703	102416003	C/T	0.047	0.036	0.434
rs3732130	6705	102416005	C/T			
rs3917329	7963	102417263	G/T	0.070	0.081	0.473
rs3917330	8525	102417825	G/T			
rs3917331	8526	102417826	A/T			
rs3917344	8598	102417898	C/T			
rs3917332	8624	102417924	A/T	0.224	0.209	0.473
rs3917333	8883	102418183	A/T			
rs3917334	8980	102418280	G/T			
rs1030021	13578	102422878	G/T	0.160	0.183	0.255
rs2241132	16135	102425435	G/T	0.604	0.631	0.385
rs2241131	16141	102425441	G/T	0.451	0.477	0.282
rs3835036	16642	102425942	-/TGG	0.424	0.463	0.112
rs1997504	16931	102426231	A/G			
rs1805232	17004	102426304	A/G			
rs1971696	17009	102426309	C/T			
rs1971695	17010	102426310	A/G			
rs3771199	18713	102428013	C/T	0.299	0.291	0.726
rs1922303	18853	102428153	C/T			
rs3213734	20783	102430083	C/T	0.826	0.860	0.099
rs1997503	21335	102430635	A/G	0.830	0.806	0.281
rs1558649	22180	102431480	C/T			
rs1558648	22268	102431568	A/C	0.127	0.142	0.439
rs1558647	22285	102431585	C/T	0.824	0.825	0.955
rs1558646	25378	102434678	C/T	0.576	0.580	0.886
rs1882514	25906	102435206	C/G	0.547	0.556	0.730
rs1882513	26015	102435315	A/G	0.500	0.513	0.574
rs867770	26475	102435775	A/G			
rs2310235	26798	102436098	A/T	0.608	0.573	0.252
rs870684	27042	102436342	A/G	0.687	0.685	0.931
rs3771197	27649	102436949	A/G	0.534	0.544	0.676
rs3771196	27827	102437127	A/T	0.171	0.189	0.558
rs3821207	27873	102437173	A/G	0.029	0.033	0.751
rs3771195	28122	102437422	A/G	0.342	0.326	0.480
rs3771194	28202	102437502	A/G	0.474	0.465	0.725
rs3771193	28232	102437532	A/C			
rs3771192	28240	102437540	G/T			
rs3755290	29546	102438846	G/T	0.348	0.329	0.428
rs3821206	29748	102439048	A/G	0.914	0.920	0.803
rs2302623	30054	102439354	A/T	0.261	0.263	0.948

dbSNP rs#	Position in SEQ ID NO: 2	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs3755289	30646	102439946	G/T	0.429	0.442	0.621
rs1922302	31149	102440449	A/C	0.574	0.539	0.166
rs2110725	36912	102446212	A/C			
rs1465326	36936	102446236	C/G	0.592	0.613	0.413
rs2871458	37184	102446484	C/T	0.068	0.059	0.549
rs2080310	39064	102448364	C/T	0.258	0.256	0.926
rs1922289	39343	102448643	G/T	0.593	0.593	0.976
rs1922290	40868	102450168	C/G	0.577	0.595	0.489
rs1922291	40917	102450217	A/G	0.344	0.358	0.549
rs1922292	41113	102450413	A/C	0.226	0.221	0.874
rs3815517	47343	102456643	A/T	0.291	0.291	0.984
rs2241130	47806	102457106	A/G	0.112	0.088	0.153
rs1922295	47911	102457211	A/G	0.362	0.349	0.594
rs1922294	48009	102457309	C/T	0.075	0.065	0.581
rs2302622	48621	102457921	C/G			
rs2310240	49245	102458545	C/G			
rs1024792	49247	102458547	C/G			
rs3836112	49299	102458599	-/CTCT	0.374	0.360	0.568
rs3074969	49302	102458602	-/AGAG	0.361	0.353	0.747
rs917994	49514	102458814	C/T	0.289	0.304	0.544
rs2041753	49626	102458926	G/T	0.330	0.329	0.981
rs2041752	49791	102459091	A/G	0.492	0.528	0.176
rs1024791	50010	102459310	A/G			
rs1024790	50294	102459594	A/G	0.771	0.776	0.828
rs995515	51482	102460782	A/G/T	0.312	0.310	0.917
rs995514	51556	102460856	A/G	0.393	0.420	0.246
rs1922293	51855	102461155	A/G	0.597	0.608	0.653
rs3755287	51956	102461256	C/T	0.869	0.885	0.458
rs3729564	52155	102461455	A/G	0.331	0.315	0.511
rs3771188	52448	102461748	A/G			
rs3771187	52458	102461758	C/T	0.280	0.258	0.332
rs3771186	52511	102461811	C/T	0.764	0.813	0.048
rs3771185	52607	102461907	A/G	0.429	0.395	0.160
rs2310241	54049	102463349	A/C	0.424	0.406	0.462
rs2302621	54224	102463524	A/C	0.323	0.340	0.473
rs2302620	54567	102463867	A/G	0.103	0.092	0.512
rs3771184	55052	102464352	C/T	0.779	0.809	0.173
rs3834161	55857	102465157	-/C	0.062	0.069	0.674
rs3755286	55941	102465241	C/G	0.786	0.817	0.150
rs3755285	56120	102465420	A/G	0.184	0.174	0.619
rs1997502	56349	102465649	C/T	0.580	0.564	0.559
rs3771182	56727	102466027	A/G	0.101	0.085	0.352
rs3836111	57232	102466532	-/CT	0.138	0.113	0.154
rs3771181	58806	102468106	C/T			
rs955754	61181	102470481	C/T	0.194	0.172	0.291
rs2302612	63808	102473108	A/G	0.135	0.120	0.456
rs3755284	64526	102473826	A/T	0.757	0.789	0.141
rs3821205	64865	102474165	A/G	0.831	0.832	0.992
rs3815511	64928	102474228	C/T	0.022	untyped	NA
rs2287041	64966	102474266	A/C	0.118	0.100	0.346
rs2287040	65080	102474380	A/G	0.518	0.536	0.462
rs2287039	65690	102474990	C/T	0.975	0.970	0.752
rs3755283	66228	102475528	A/G			
rs3755282	66982	102476282	A/G	0.312	0.295	0.452
rs1812326	72511	102481811	A/G	0.343	0.297	0.054
rs1558626	74170	102483470	A/T	0.536	0.551	0.643

dbSNP rs#	Position in SEQ ID NO: 2	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs1558625	74264	102483564	C/T	0.661	0.697	0.128
rs1558624	74333	102483633	C/T	0.322	0.278	0.074
rs1558623	74502	102483802	A/T	0.303	0.273	0.200
rs1035131	74741	102484041	A/C	0.543	0.595	0.046
rs2110661	75321	102484621	C/T	0.430	0.413	0.485
rs1420093	82558	102491858	A/G	0.381	0.388	0.826
rs3074971	85366	102494666	-/TTG	0.438	0.479	0.096
rs1345302	85469	102494769	C/T	0.428	0.397	0.223
rs1420092	86485	102495785	G/T	0.792	0.793	0.965
rs1345301	87687	102496987	C/T	0.514	0.477	0.131
rs2310242	89463	102498763	G/T	0.108	0.114	0.804
rs2310243	89660	102498960	A/G	0.490	0.523	0.194
rs1882510	95718	102505018	C/T	0.617	0.667	0.075
rs1882511	95821	102505121	A/G	0.664	0.652	0.599

[0237] The IL1RL2 proximal SNPs were also allelotyped in the replication cohorts using the methods described herein and the primers provided in Tables 11 and 12. The replication allelotyping results for replication cohort #1 and replication cohort #2 are provided in Tables 14 and 15, respectively.

TABLE 14

dbSNP rs#	Position in SEQ ID NO: 2	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs3917304	225	102409525	G/T	0.599	0.592	0.843
rs2041747	509	102409809	C/T	0.021	0.026	0.845
rs3917305	860	102410160	C/T			
rs3771200	874	102410174	C/T	0.442	0.482	0.207
rs3917306	939	102410239	A/G			
rs3917307	1483	102410783	G/T			
rs3917308	1798	102411098	C/T			
rs3917310	2189	102411489	A/T			
rs3917311	2215	102411515	A/G	0.933	0.974	0.042
rs3917312	2282	102411582	C/G			
rs3917313	2340	102411640	C/T			
rs3917314	2963	102412263	A/C	0.036	0.038	0.918
rs3917316	3369	102412669	-/T	0.904	0.963	0.072
rs3171845	3481	102412781	A/G	0.898	0.882	0.610
rs3171846	3564	102412864	G/T			
rs3917317	3653	102412953	-/TC	0.313	0.323	0.759
rs3917318	4860	102414160	A/G	0.149	0.142	0.803
rs3917319	4941	102414241	A/T			
rs3917320	4975	102414275	A/C	0.921	0.930	0.749
rs3917321	5321	102414621	A/G			
rs3917322	5346	102414646	A/G			
rs3917323	5541	102414841	A/G			
rs3917324	5633	102414933	C/G			
rs3917325	6007	102415307	G/T	0.033	0.040	0.716
rs3732134	6317	102415617	C/G			
rs3732133	6378	102415678	A/G			
rs2110726	6382	102415682	C/T	0.334	0.339	0.880
rs3917326	6426	102415726	C/T			
rs3917327	6479	102415779	C/G			

dbSNP rs#	Position in SEQ ID NO: 2	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs3917328	6641	102415941	C/T	0.885	0.867	0.523
rs3732131	6703	102416003	C/T	0.045	0.022	0.224
rs3732130	6705	102416005	C/T			
rs3917329	7963	102417263	G/T	0.068	0.091	0.296
rs3917330	8525	102417825	G/T			
rs3917331	8526	102417826	A/T			
rs3917344	8598	102417898	C/T			
rs3917332	8624	102417924	A/T	0.203	0.195	0.785
rs3917333	8883	102418183	A/T			
rs3917334	8980	102418280	G/T			
rs1030021	13578	102422878	G/T	0.148	0.174	0.325
rs2241132	16135	102425435	G/T	0.604	0.595	0.815
rs2241131	16141	102425441	G/T	0.452	0.464	0.696
rs3835036	16642	102425942	-TGG	0.402	0.479	0.017
rs1997504	16931	102426231	A/G			
rs1805232	17004	102426304	A/G			
rs1971696	17009	102426309	C/T			
rs1971695	17010	102426310	A/G			
rs3771199	18713	102428013	C/T	0.317	0.310	0.818
rs1922303	18853	102428153	C/T			
rs3213734	20783	102430083	C/T	0.824	0.892	0.012
rs1997503	21335	102430635	A/G	0.838	0.790	0.114
rs1558649	22180	102431480	C/T			
rs1558648	22268	102431568	A/C	0.125	0.164	0.121
rs1558647	22285	102431585	C/T	0.834	0.831	0.895
rs1558646	25378	102434678	C/T	0.547	0.561	0.672
rs1882514	25906	102435206	C/G	0.538	0.542	0.905
rs1882513	26015	102435315	A/G	0.471	0.497	0.414
rs867770	26475	102435775	A/G			
rs2310235	26798	102436098	A/T	0.562	NA	0.608
rs870684	27042	102436342	A/G	0.657	0.680	0.509
rs3771197	27649	102436949	A/G	0.502	0.534	0.351
rs3771196	27827	102437127	A/T	0.171	0.189	0.558
rs3821207	27873	102437173	A/G	0.033	0.038	0.821
rs3771195	28122	102437422	A/G	0.374	0.342	0.311
rs3771194	28202	102437502	A/G	0.493	0.480	0.696
rs3771193	28232	102437532	A/C			
rs3771192	28240	102437540	G/T			
rs3755290	29546	102438846	G/T	0.364	0.346	0.602
rs3821206	29748	102439048	A/G	0.940	NA	0.914
rs2302623	30054	102439354	A/T	0.267	0.268	0.984
rs3755289	30646	102439946	G/T	0.417	0.451	0.281
rs1922302	31149	102440449	A/C	0.600	0.559	0.245
rs2110725	36912	102446212	A/C			
rs1465326	36936	102446236	C/G	0.573	0.614	0.296
rs2871458	37184	102446484	C/T	0.085	0.070	0.530
rs2080310	39064	102448364	C/T	0.277	0.268	0.776
rs1922289	39343	102448643	G/T	0.580	0.576	0.924
rs1922290	40868	102450168	C/G	0.558	0.579	0.556
rs1922291	40917	102450217	A/G	0.322	0.348	0.401
rs1922292	41113	102450413	A/C	0.235	untyped	NA
rs3815517	47343	102456643	A/T	0.310	0.312	0.950
rs2241130	47806	102457106	A/G	0.110	0.068	0.071
rs1922295	47911	102457211	A/G	0.378	0.364	0.695
rs1922294	48009	102457309	C/T	0.061	0.055	0.799
rs2302622	48621	102457921	C/G			

dbSNP rs#	Position in SEQ ID NO: 2	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs2310240	49245	102458545	C/G			
rs1024792	49247	102458547	C/G			
rs3836112	49299	102458599	-/CTCT	0.407	0.378	0.382
rs3074969	49302	102458602	-/AGAG	0.385	0.362	0.497
rs917994	49514	102458814	C/T	0.271	0.281	0.757
rs2041753	49626	102458926	G/T	0.357	0.342	0.672
rs2041752	49791	102459091	A/G	0.459	0.511	0.155
rs1024791	50010	102459310	A/G			
rs1024790	50294	102459594	A/G	0.781	0.773	0.769
rs995515	51482	102460782	A/G/T	0.331	0.323	0.825
rs995514	51556	102460856	A/G	0.373	0.412	0.221
rs1922293	51855	102461155	A/G	0.568	0.597	0.376
rs3755287	51956	102461256	C/T	0.867	0.907	0.138
rs3729564	52155	102461455	A/G	0.362	0.320	0.212
rs3771188	52448	102461748	A/G			
rs3771187	52458	102461758	C/T	0.308	0.276	0.288
rs3771186	52511	102461811	C/T	0.761	0.847	0.003
rs3771185	52607	102461907	A/G	0.445	0.385	0.069
rs2310241	54049	102463349	A/C	0.446	0.400	0.161
rs2302621	54224	102463524	A/C	0.304	0.326	0.499
rs2302620	54567	102463867	A/G	0.100	0.074	0.236
rs3771184	55052	102464352	C/T	0.785	0.853	0.014
rs3834161	55857	102465157	-/C	0.068	0.081	0.596
rs3755286	55941	102465241	C/G	0.791	0.850	0.038
rs3755285	56120	102465420	A/G	0.194	0.173	0.446
rs1997502	56349	102465649	C/T	0.604	0.577	0.536
rs3771182	56727	102466027	A/G	0.107	0.070	0.117
rs3836111	57232	102466532	-/CT	0.137	0.090	0.048
rs3771181	58806	102468106	C/T			
rs955754	61181	102470481	C/T	0.209	0.160	0.084
rs2302612	63808	102473108	A/G	0.138	0.111	0.331
rs3755284	64526	102473826	A/T	0.754	0.829	0.010
rs3821205	64865	102474165	A/G	0.799	0.814	0.594
rs3815511	64928	102474228	C/T			
rs2287041	64966	102474266	A/C	0.113	0.074	0.143
rs2287040	65080	102474380	A/G	0.493	0.521	0.386
rs2287039	65690	102474990	C/T	0.970	0.962	0.703
rs3755283	66228	102475528	A/G			
rs3755282	66982	102476282	A/G	0.327	0.312	0.636
rs1812326	72511	102481811	A/G	0.362	0.299	0.067
rs1558626	74170	102483470	A/T	0.558	untyped	
rs1558625	74264	102483564	C/T	0.635	0.683	0.137
rs1558624	74333	102483633	C/T	0.350	0.278	0.024
rs1558623	74502	102483802	A/T	0.323	0.281	0.204
rs1035131	74741	102484041	A/C	0.513	0.598	0.026
rs2110661	75321	102484621	C/T	0.449	0.412	0.237
rs1420093	82558	102491858	A/G	0.390	untyped	
rs3074971	85366	102494666	-/TTG	0.398	0.485	0.006
rs1345302	85469	102494769	C/T	0.468	0.392	0.036
rs1420092	86485	102495785	G/T	0.810	0.808	0.958
rs1345301	87687	102496987	C/T	0.554	0.470	0.016
rs2310242	89463	102498763	G/T	0.110	untyped	
rs2310243	89660	102498960	A/G	0.452	0.529	0.031
rs1882510	95718	102505018	C/T	0.597	0.688	0.022
rs1882511	95821	102505121	A/G	0.684	0.657	0.373

TABLE 15

dbSNP rs#	Position in Figure 2	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs3917304	225	102409525	G/T	0.531	0.483	0.236
rs2041747	509	102409809	C/T	0.034	untyped	
rs3917305	860	102410160	C/T			
rs3771200	874	102410174	C/T	0.500	0.460	0.282
rs3917306	939	102410239	A/G			
rs3917307	1483	102410783	G/T			
rs3917308	1798	102411098	C/T			
rs3917310	2189	102411489	A/T			
rs3917311	2215	102411515	A/G	0.959	0.947	0.574
rs3917312	2282	102411582	C/G			
rs3917313	2340	102411640	C/T			
rs3917314	2963	102412263	A/C			
rs3917316	3369	102412669	-/T	0.633	0.687	0.176
rs3171845	3481	102412781	A/G	0.912	0.913	0.964
rs3171846	3564	102412864	G/T			
rs3917317	3653	102412953	-/TC	0.329	0.329	0.999
rs3917318	4860	102414160	A/G	0.153	0.165	0.696
rs3917319	4941	102414241	A/T			
rs3917320	4975	102414275	A/C	0.955	0.971	0.463
rs3917321	5321	102414621	A/G			
rs3917322	5346	102414646	A/G			
rs3917323	5541	102414841	A/G			
rs3917324	5633	102414933	C/G			
rs3917325	6007	102415307	G/T	0.023	untyped	
rs3732134	6317	102415617	C/G			
rs3732133	6378	102415678	A/G			
rs2110726	6382	102415682	C/T	0.301	0.285	0.632
rs3917326	6426	102415726	C/T			
rs3917327	6479	102415779	C/G			
rs3917328	6641	102415941	C/T	0.915	0.929	0.621
rs3732131	6703	102416003	C/T	0.049	0.058	0.670
rs3732130	6705	102416005	C/T			
rs3917329	7963	102417263	G/T	0.073	0.067	0.798
rs3917330	8525	102417825	G/T			
rs3917331	8526	102417826	A/T			
rs3917344	8598	102417898	C/T			
rs3917332	8624	102417924	A/T	0.251	0.231	0.534
rs3917333	8883	102418183	A/T			
rs3917334	8980	102418280	G/T			
rs1030021	13578	102422878	G/T	0.176	0.197	0.489
rs2241132	16135	102425435	G/T	untyped	0.688	NA
rs2241131	16141	102425441	G/T	0.451	0.498	0.204
rs3835036	16642	102425942	-/TGG	0.453	0.439	0.715
rs1997504	16931	102426231	A/G			
rs1805232	17004	102426304	A/G			
rs1971696	17009	102426309	C/T			
rs1971695	17010	102426310	A/G			
rs3771199	18713	102428013	C/T	0.277	0.262	0.665
rs1922303	18853	102428153	C/T			
rs3213734	20783	102430083	C/T	0.827	0.809	0.573
rs1997503	21335	102430635	A/G	0.821	0.832	0.740
rs1558649	22180	102431480	C/T			
rs1558648	22268	102431568	A/C	0.130	0.105	0.368
rs1558647	22285	102431585	C/T	0.810	0.815	0.861

dbSNP rs#	Position in Figure 2	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs1558646	25378	102434678	C/T	0.613	0.608	0.893
rs1882514	25906	102435206	C/G	0.558	0.578	0.630
rs1882513	26015	102435315	A/G	0.537	0.539	0.952
rs867770	26475	102435775	A/G			
rs2310235	26798	102436098	A/T	0.589	0.019	
rs870684	27042	102436342	A/G	0.726	0.693	0.392
rs3771197	27649	102436949	A/G	0.574	0.561	0.725
rs3771196	27827	102437127	A/T			
rs3821207	27873	102437173	A/G	0.023	0.026	0.884
rs3771195	28122	102437422	A/G	0.303	0.301	0.952
rs3771194	28202	102437502	A/G	0.450	0.442	0.832
rs3771193	28232	102437532	A/C			
rs3771192	28240	102437540	G/T			
rs3755290	29546	102438846	G/T	0.328	0.302	0.452
rs3821206	29748	102439048	A/G	0.889	0.026	
rs2302623	30054	102439354	A/T	0.254	0.255	0.962
rs3755289	30646	102439946	G/T	0.444	0.429	0.744
rs1922302	31149	102440449	A/C	0.541	0.507	0.364
rs2110725	36912	102446212	A/C			
rs1465326	36936	102446236	C/G	0.616	0.612	0.919
rs2871458	37184	102446484	C/T	0.046	0.041	0.775
rs2080310	39064	102448364	C/T	0.235	0.238	0.933
rs1922289	39343	102448643	G/T	0.611	0.618	0.845
rs1922290	40868	102450168	C/G	0.601	0.619	0.631
rs1922291	40917	102450217	A/G	0.372	0.374	0.961
rs1922292	41113	102450413	A/C	0.215	0.221	0.827
rs3815517	47343	102456643	A/T	0.268	0.257	0.766
rs2241130	47806	102457106	A/G	0.115	0.119	0.854
rs1922295	47911	102457211	A/G	0.342	0.325	0.632
rs1922294	48009	102457309	C/T	0.092	0.081	0.677
rs2302622	48621	102457921	C/G			
rs2310240	49245	102458545	C/G			
rs1024792	49247	102458547	C/G			
rs3836112	49299	102458599	-/CTCT	0.332	0.332	0.999
rs3074969	49302	102458602	-/AGAG	0.330	0.339	0.822
rs917994	49514	102458814	C/T	0.312	0.339	0.456
rs2041753	49626	102458926	G/T	0.296	0.310	0.737
rs2041752	49791	102459091	A/G	0.534	0.556	0.587
rs1024791	50010	102459310	A/G			
rs1024790	50294	102459594	A/G	0.759	0.780	0.498
rs995515	51482	102460782	A/G/T	0.288	0.288	0.992
rs995514	51556	102460856	A/G	0.417	0.434	0.657
rs1922293	51855	102461155	A/G	0.634	0.625	0.806
rs3755287	51956	102461256	C/T	0.873	0.850	0.471
rs3729564	52155	102461455	A/G	0.291	0.308	0.643
rs3771188	52448	102461748	A/G			
rs3771187	52458	102461758	C/T	0.246	0.231	0.677
rs3771186	52511	102461811	C/T	0.766	0.759	0.850
rs3771185	52607	102461907	A/G	0.409	0.410	0.972
rs2310241	54049	102463349	A/C	0.396	0.416	0.591
rs2302621	54224	102463524	A/C	0.347	0.363	0.667
rs2302620	54567	102463867	A/G	0.107	0.121	0.605
rs3771184	55052	102464352	C/T	0.772	0.740	0.364
rs3834161	55857	102465157	-/C	0.054	0.051	0.860
rs3755286	55941	102465241	C/G	0.781	0.766	0.641
rs3755285	56120	102465420	A/G	0.172	0.175	0.897
rs1997502	56349	102465649	C/T	0.550	0.543	0.849

dbSNP rs#	Position in Figure 2	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs3771182	56727	102466027	A/G	0.094	0.109	0.562
rs3836111	57232	102466532	-/CT	0.139	0.148	0.750
rs3771181	58806	102468106	C/T			
rs955754	61181	102470481	C/T	0.173	0.190	0.571
rs2302612	63808	102473108	A/G	0.132	0.135	0.909
rs3755284	64526	102473826	A/T	0.760	0.726	0.332
rs3821205	64865	102474165	A/G	0.873	0.859	0.629
rs3815511	64928	102474228	C/T			
rs2287041	64966	102474266	A/C	0.124	0.141	0.517
rs2287040	65080	102474380	A/G	0.550	0.559	0.802
rs2287039	65690	102474990	C/T			
rs3755283	66228	102475528	A/G			
rs3755282	66982	102476282	A/G	0.293	0.268	0.452
rs1812326	72511	102481811	A/G	0.320	0.294	0.453
rs1558626	74170	102483470	A/T	0.541	untyped	
rs1558625	74264	102483564	C/T	0.694	0.719	0.473
rs1558624	74333	102483633	C/T	0.285	0.279	0.865
rs1558623	74502	102483802	A/T	0.277	0.261	0.615
rs1035131	74741	102484041	A/C	0.581	0.590	0.795
rs2110661	75321	102484621	C/T	0.405	0.414	0.800
rs1420093	82558	102491858	A/G	0.384	untyped	
rs3074971	85366	102494666	-/TTG	0.488	0.469	0.619
rs1345302	85469	102494769	C/T	0.378	0.406	0.437
rs1420092	86485	102495785	G/T	0.769	0.768	0.980
rs1345301	87687	102496987	C/T	0.464	0.487	0.531
rs2310242	89463	102498763	G/T	0.120	untyped	
rs2310243	89660	102498960	A/G	0.537	0.514	0.548
rs1882510	95718	102505018	C/T	0.642	0.635	0.875
rs1882511	95821	102505121	A/G	0.639	0.644	0.871

[0238] Allelotyping results were considered particularly significant with a calculated p-value of less than or equal to 0.05 for allelotype results. These values are indicated in bold. The allelotyping p-values were plotted in Figure 1B for the discovery cohort. The position of each SNP on the chromosome is presented on the x-axis. The y-axis gives the negative logarithm (base 10) of the p-value comparing the estimated allele in the case group to that of the control group. The minor allele frequency of the control group for each SNP designated by an X or other symbol on the graphs in Figure 1B can be determined by consulting Table 13. For example, the left-most X on the left graph is at position 102409525. By proceeding down the Table from top to bottom and across the graphs from left to right the allele frequency associated with each symbol shown can be determined.

[0239] To aid the interpretation, multiple lines have been added to the graph. The broken horizontal lines are drawn at two common significance levels, 0.05 and 0.01. The vertical broken lines are drawn every 20kb to assist in the interpretation of distances between SNPs. Two other lines are drawn to expose linear trends in the association of SNPs to the disease. The light gray line (or generally bottom-most curve) is a nonlinear smoother through the data points on the graph using a local polynomial

regression method (W.S. Cleveland, E. Grosse and W.M. Shyu (1992) Local regression models. Chapter 8 of Statistical Models in S eds J.M. Chambers and T.J. Hastie, Wadsworth & Brooks/Cole.). The black line provides a local test for excess statistical significance to identify regions of association. This was created by use of a 10kb sliding window with 1kb step sizes. Within each window, a chi-square goodness of fit test was applied to compare the proportion of SNPs that were significant at a test wise level of 0.01, to the proportion that would be expected by chance alone (0.05 for the methods used here). Resulting p-values that were less than 10^{-8} were truncated at that value.

[0240] Finally, the exons and introns of the genes in the covered region are plotted below each graph at the appropriate chromosomal positions. The gene boundary is indicated by the broken horizontal line. The exon positions are shown as thick, unbroken bars. An arrow is placed at the 3' end of each gene to show the direction of transcription.

Example 5

WASPIP Region Proximal SNPs

[0241] It has been discovered that rs1465621 in the untranslated region (UTR) of the WASPIP gene is associated with occurrence of osteoarthritis in subjects. This gene encodes a protein that plays a role in actin cytoskeleton organization. The encoded protein binds to a region of Wiskott-Aldrich syndrome protein that is frequently mutated in Wiskott-Aldrich syndrome, an X-linked recessive disorder. Impairment of the interaction between these two proteins may contribute to the disease. Alternative transcript variants exist for this gene. Biological activity of WASPIP or a pathway member downstream of WASPIP (e.g., IL-2) can be modulated by addition of an antibody, a recombinant binding partner, a binding agent, or a recombinant WASPIP or downstream pathway member protein or functional fragment thereof.

[0242] Sixty-one additional allelic variants proximal to rs1465621 were identified and subsequently allelotyped in osteoarthritis case and control sample sets as described in Examples 1 and 2. The polymorphic variants are set forth in Table 16. The chromosome positions provided in column four of Table 16 are based on Genome "Build 34" of NCBI's GenBank.

TABLE 16

dbSNP rs#	Chromosome	Position in SEQ ID NO: 3	Chromosome Position	Allele Variants
rs1864455	2	209	175603909	C/T
rs1971763	2	5908	175609608	C/T
rs934269	2	7460	175611160	A/G
rs934270	2	7733	175611433	A/G
rs2033309	2	7855	175611555	A/G

dbSNP rs#	Chromosome	Position in SEQ ID NO: 3	Chromosome Position	Allele Variants
rs2033310	2	7904	175611604	A/C
rs934271	2	8869	175612569	G/T
rs934272	2	9480	175613180	C/T
rs1897110	2	13820	175617520	C/T
rs2033311	2	15152	175618852	A/G
rs1010027	2	17713	175621413	A/G
rs1010028	2	17804	175621504	C/T
rs2884502	2	18220	175621920	C/T
rs1430177	2	19083	175622783	C/T
rs1430178	2	19123	175622823	C/G
rs3043779	2	19605	175623305	-/GTAAA
rs1549742	2	20247	175623947	G/T
rs3043781	2	20592	175624292	-/CCCCC
rs2033313	2	21907	175625607	C/T
rs7739	2	23273	175626973	C/T
rs11482	2	23299	175626999	A/C
rs3087907	2	23623	175627323	G/T
rs2358888	2	23669	175627369	A/T
rs1046036	2	23844	175627544	A/T
rs3205060	2	24190	175627890	A/G
rs15327	2	24486	175628186	C/T
rs1430179	2	24896	175628596	A/C
rs1430180	2	25118	175628818	C/G
rs2163236	2	30551	175634251	C/G
rs3217351	2	30844	175634544	-/GAGA
rs2303891	2	30900	175634600	A/G
rs3815969	2	30942	175634642	A/G
rs2288622	2	31699	175635399	A/G
rs2288623	2	32081	175635781	G/T
rs1044335	2	35078	175638778	A/G
rs2288624	2	36196	175639896	A/T
rs1060511	2	36541	175640241	A/C
rs1367218	2	38356	175642056	A/G
rs1367217	2	45578	175649278	A/G
rs1465621	2	49634	175653334	A/T
rs1465622	2	49774	175653474	G/T
rs2115872	2	51119	175654819	A/G
rs1465623	2	51181	175654881	A/G
rs1469521	2	51652	175655352	C/T
rs1864451	2	54467	175658167	C/G
rs1430183	2	55762	175659462	A/G
rs1430182	2	55999	175659699	A/G
rs1430181	2	57865	175661565	A/C
rs1991601	2	66613	175670313	A/G
rs2358890	2	68377	175672077	C/T
rs2115875	2	69754	175673454	C/T

dbSNP rs#	Chromosome	Position in SEQ ID NO: 3	Chromosome Position	Allele Variants
rs1430185	2	72859	175676559	A/G
rs2217429	2	76512	175680212	A/G
rs3049909	2	76717	175680417	-/AT
rs1430184	2	77722	175681422	C/T
rs2278321	2	80998	175684698	A/G
rs2115874	2	82033	175685733	C/T
rs2033315	2	89658	175693358	C/T
rs2033314	2	89960	175693660	A/G
rs1991600	2	94155	175697855	A/G
rs1864453	2	95679	175699379	A/G

Assay for Verifying and Allelotyping SNPs

[0243] The methods used to verify and allelotype the 61 proximal SNPs of Table 16 are the same methods described in Examples 1 and 2 herein. The primers and probes used in these assays are provided in Table 17 and Table 18, respectively.

TABLE 17

dbSNP rs#	Forward PCR primer	Reverse PCR primer
rs1864455	ACGTTGGATGACAGGTGTGCAGTGAATGTC	ACGTTGGATGTCAGCAGTTGTCCCATCTTC
rs1971763	ACGTTGGATGAATGATTTACTTGAGGCCGG	ACGTTGGATGTCTCAAACCTGACCTCTG
rs934269	ACGTTGGATGAAGTCCCTAGGACTACAGGT	ACGTTGGATGTGGGCAACATAGCAAGACCC
rs934270	ACGTTGGATGATGATCTGCCCTGTTCTTGC	ACGTTGGATGAGGTGCAATCTACTCACCAG
rs2033309	ACGTTGGATGCCATAGCTTCTCACAAC	ACGTTGGATGTTCTCCTTGACAGACAAGGTG
rs2033310	ACGTTGGATGATGAGTCTCTGTGAGTTGAG	ACGTTGGATGTTGTGTGAGGAAGCTATGGC
rs934271	ACGTTGGATGCCTGAAATGCCAAGAAGAATG	ACGTTGGATGATTCTTGCTACATAGTCAGG
rs934272	ACGTTGGATGAGTCTTGCTTCTCTTACAC	ACGTTGGATGACTAAGAGGTATTTGGGTGC
rs1897110	ACGTTGGATGTCAGCATCCCAAAGTGCTAG	ACGTTGGATGTAAAAATCGGCTGGGTGTGG
rs2033311	ACGTTGGATGCGGGACTCTGTGTTAACAAG	ACGTTGGATGGAGTTACAAGATGCTGGAGC
rs1010027	ACGTTGGATGGCCGTCTCTGTTGTGAGAAG	ACGTTGGATGAATTCCTCTCTGACTCTTTC
rs1010028	ACGTTGGATGGTAACTTAAGGCCTCACAGC	ACGTTGGATGGACTGAAAGAGTCAGAGAGG
rs2884502	ACGTTGGATGGAATCCCATGTCAGAATC	ACGTTGGATGTGAACAGTACAAAGGAAGGG
rs1430177	ACGTTGGATGGCCAGACCCTGTCTCAAATA	ACGTTGGATGTGAGTAGCTAGGAGTATAGG
rs1430178	ACGTTGGATGTATTTGAGACAGGGTCTGGC	ACGTTGGATGTGAGCCCTGGAATTCAGAC
rs3043779	ACGTTGGATGAGTTCCTCAACTACTGTTT	ACGTTGGATGCCACATGATTTAATGGAGC
rs1549742	ACGTTGGATGTGAGACACTGTGCCTAGCTG	ACGTTGGATGGGTCCAGGTTTTGTGATGTC
rs3043781	ACGTTGGATGATAATAAATAGTTAGAAGCC	ACGTTGGATGAGAAGCTAATTAAGCTCAAG
rs2033313	ACGTTGGATGAAGCCGTGCACTCACAAATC	ACGTTGGATGACCACCTACAAAGCTTCTGG
rs7739	ACGTTGGATGTGATGACACAGATAGCAAATGTG	ACGTTGGATGTTCCCTCCTTATAGTCAAGGACC
rs11482	ACGTTGGATGAAATGTTGGCATGAAATTAATTTT	ACGTTGGATGTGTGTCTGTTTACATAGTGCATG
rs3087907	ACGTTGGATGGAACACTGAGTTTAACTG	ACGTTGGATGAATCAGAGCTTACATGTGTG

dbSNP rs#	Forward PCR primer	Reverse PCR primer
rs2358888	ACGTTGGATGAATCAGAGCTTACATGTGTG	ACGTTGGATGGAGGTGAATGTTAAATACTG
rs1046036	ACGTTGGATGCAAAGTTGCCATTATCCAG	ACGTTGGATGAGGGGTAGGTGTATTAATG
rs3205060	ACGTTGGATGAAGCCAACACTTTGCCAAGC	ACGTTGGATGTCCTCTCTCTCTACCATTG
rs15327	ACGTTGGATGGGGTTGGTTTCTTGGTAGCA	ACGTTGGATGCCTAAACATTGTATCATGGTTTCA
rs1430179	ACGTTGGATGAGACTAGGAAGGCTTGGTAG	ACGTTGGATGGGTTCCCTTCTTCTTCCATG
rs1430180	ACGTTGGATGCTTCAAAGTACCAAGGTCAG	ACGTTGGATGCAGGCTTTCATTGTGTTCC
rs2163236	ACGTTGGATGTTGAGTAGCCTGAGTGACAC	ACGTTGGATGTAGATGGCTCCAAAGGGTTC
rs3217351	ACGTTGGATGGTAACGAAAGGCACAGAATG	ACGTTGGATGTAGCACTTCCAGCTTTTCTG
rs2303891	ACGTTGGATGACCACAGACATCAGTGCTAG	ACGTTGGATGCAGTGTACTAATTCGTGACC
rs3815969	ACGTTGGATGGAAGTGCTACAAAGGTCACG	ACGTTGGATGGCTGGATCCTAATCACTCTC
rs2288622	ACGTTGGATGGGCCTGGAGCAAAAAAGAC	ACGTTGGATGCATCAGCTGTACACCAATGG
rs2288623	ACGTTGGATGGAATTAATTTAGGTCTTCAG	ACGTTGGATGTATACATCACAGAAACATGC
rs1044335	ACGTTGGATGCTACTCAGTGTCTCATCTC	ACGTTGGATGTTAAGTGGCACACGACACG
rs2288624	ACGTTGGATGCATAGGCTGTAGAAGTTGGG	ACGTTGGATGTTGTTGGTCTTCTTGGGAG
rs1060511	ACGTTGGATGTCCTATGAAGAGAAATGCC	ACGTTGGATGCTGATGGTCTTTTCTCTTC
rs1367218	ACGTTGGATGTTGTGAGCCGCTTTTCAAAC	ACGTTGGATGCATGCAAAACACTTTTTCAG
rs1367217	ACGTTGGATGGAGCTGTAATAAAAGGGTG	ACGTTGGATGGTGTATATTGCCAAAGATGC
rs1465621	ACGTTGGATGTTCTCCTCCCATTCTTCTG	ACGTTGGATGGCGGGACTAGAAGTAGATTG
rs1465622	ACGTTGGATGGGTCTTTGAGTGCTCCAAAC	ACGTTGGATGAGATGTCAGGTGGAAAGCA
rs2115872	ACGTTGGATGTAGACCGCCCACTTTGAATG	ACGTTGGATGAAGACACTGCTGGACTTGTG
rs1465623	ACGTTGGATGGGATCCAGCAGATTCTCCAT	ACGTTGGATGAGTGGCGGCTAGAAAATG
rs1469521	ACGTTGGATGTGGTCTAGGAGACGTCTGA	ACGTTGGATGAGGACTGGGTGCCTGTGTTA
rs1864451	ACGTTGGATGCTGTATGTGAAAACAAAAGCC	ACGTTGGATGTCTTTACTTGGTGTGTTGAC
rs1430183	ACGTTGGATGCATGTCATTCTGTAGTGTGG	ACGTTGGATGTCCTTGGGATCAAGAAAGTG
rs1430182	ACGTTGGATGAATGTTGCTAAAAGTAACCC	ACGTTGGATGATCTTTTGGGAAAAGAAG
rs1430181	ACGTTGGATGAAGCTCCTAGCCAGTCTTAG	ACGTTGGATGTTATTTGGCGGGGAGTAGG
rs1991601	ACGTTGGATGATCCTCAACAGATCTGGTTC	ACGTTGGATGTCTGGTGATGGCTTGTGATC
rs2358890	ACGTTGGATGTCAGAGTAGAGTTACTCCAG	ACGTTGGATGCATGATGCAGCTATTCTGTG
rs2115875	ACGTTGGATGCAGACCCTTTTTCTAGATC	ACGTTGGATGACTATTTTGAAGTAGTGTG
rs1430185	ACGTTGGATGATCTGAGCCTAGACCTTAAC	ACGTTGGATGGGGAATGAATACAACAGTGC
rs2217429	ACGTTGGATGTGCACAAAATTAGCCACAGC	ACGTTGGATGAGTGACCGTTTCTGTGTGT
rs3049909	ACGTTGGATGCAAAAGCAGGAATGCCTTGG	ACGTTGGATGGGGTCACAAGTCTGTTTTC
rs1430184	ACGTTGGATGAATTAGCAATGGCTCTCTCC	ACGTTGGATGCCTAAAAACACAGTTGCTCC
rs2278321	ACGTTGGATGCAGACAGCAGGTAGATGAAC	ACGTTGGATGTCTGGAAAAGAGAGACAGCC
rs2115874	ACGTTGGATGCAGTGGACTTAAGAGAGGAG	ACGTTGGATGGGTTACAGTACCTGAAAAGC
rs2033315	ACGTTGGATGGTCAAGGTAGTTGAGAGTATT	ACGTTGGATGCAATGACAAAAGCAATTTTC
rs2033314	ACGTTGGATGCATCTTTCTTAATGGTCTTGG	ACGTTGGATGATGCAGAGTCACATTCCATG
rs1991600	ACGTTGGATGTTTCGTATCAGTCAGAAGG	ACGTTGGATGCTGGTTCCTTTTTTGGGAG
rs1864453	ACGTTGGATGAGATAGGAATGACTGCCAAG	ACGTTGGATGAGGTGACTTCATCTCTTTCC

TABLE 18

dbSNP rs#	Extend Primer	Term Mix
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dbSNP rs#	Extend Primer	Term Mix
rs1864455	TCCTTTTCTCTCAGTTCCCC	ACT
rs1971763	AGCACTTTGGGAGGCCAAGG	ACG
rs934269	GCACGCCACCACACTCGG	ACG
rs934270	CCCTGTTCTTGCTCCTGCTTCTT	ACT
rs2033309	ACAACACAAAGAAGGGTTGTTA	ACG
rs2033310	GGGTGGGAAATCTGCTGAG	ACT
rs934271	GCATAATTTTTCAGGGAGGCAG	ACT
rs934272	TGCTTCTCTTCACACTTATAAG	ACG
rs1897110	GCATCCCAAAGTGCTAGGATTACA	ACT
rs2033311	CTTCCAGGAGGTGCGATGAG	ACT
rs1010027	TCTGTTGTGAGAAGATGCGC	ACT
rs1010028	ACAGCTGTTGGGCTCACAG	ACT
rs2884502	TGCCTAGTTAATTTGCTTTCCT	ACT
rs1430177	CCCTGTCTCAAATAAATTTTAAAA	ACT
rs1430178	GACAGGGTCTGGCTATGTTGTC	ACT
rs3043779	ACTGTTTGTTGATGATTTGAATAA	ACT
rs1549742	GCCTAGCTGGGGCTTCAAGTTA	CGT
rs3043781	TAGAAGCCAACCCCCCCC	ACT
rs2033313	CCCTGTGAGGCCATAGACAA	ACT
rs7739	CTGTTTACATAGTGCATG	ACT
rs11482	CTTATAGTCAAGGACCGT	CGT
rs3087907	CAATATAAAATAAGAGGTGAATGT	ACT
rs2358888	GCTTACATGTGTGTTTTTT	CGT
rs1046036	CATTCATCCAGAATAGATTGTTTT	CGT
rs3205060	TTTGCCAAGCTTGTTATA	ACG
rs15327	GGTAGCATCTCCAGTAA	ACG
rs1430179	GAGGGGAAAAAAGTCAGGAAAA	ACT
rs1430180	AAGTACCAAGGTCAGAAATTGATT	ACT
rs2163236	AGTCCAGGCTTCTTGCCTG	ACT
rs3217351	AGGCACAGAATGAAAGAGAGA	ACT
rs2303891	TAGAAGTTTACAGAAAAGCTGGAA	ACT
rs3815969	TTAGTACACTGACATATATACAG	ACT
rs2288622	CTTACATCCACATTCCATTACC	ACT
rs2288623	TTTTAGGTCTTCAGAAGAACAAAG	ACT
rs1044335	GAAATATTGGTCCCACTTTCC	ACG
rs2288624	GACTCGCAGGTAAATAGAGCT	CGT
rs1060511	CCCAAAAAAAGTGAAAAA	CGT
rs1367218	CTTTTCAAACACGATGGAGCAC	ACT
rs1367217	AACTAAAAAGGGTGATTTCACTAT	ACT
rs1465621	CCATTCTTCTGACATTGCGC	CGT
rs1465622	CAAACATAAGGTTGACCCCC	CGT
rs2115872	TTTGAATGGGACTCTTCC	ACT
rs1465623	TCCATACATGAGAGCTGCTG	ACG

dbSNP rs#	Extend Primer	Term Mix
rs1469521	TAGGAGACGTCTGACTCCAA	ACT
rs1864451	GAAAACAAAAGCCTTTTCTGTC	ACT
rs1430183	ATTCTGTAGTGTGGGCCCTA	ACT
rs1430182	GTAACCCTTAAATACTATCATAC	ACG
rs1430181	CTAGCCAGTCTTAGTGATGTT	CGT
rs1991601	AGCTCGCCTCAGCCTACAA	ACT
rs2358890	GTCCAGAACACCATAATCCC	ACT
rs2115875	TTTTTCTAGATCAGCACTGTTCA	ACT
rs1430185	CTAGACCTTAACTCCAATTATA	ACG
rs2217429	AGTCCTTGTTTATGAACATTTG	ACT
rs3049909	TTTATGTTATGCACATGCAGAC	ACT
rs1430184	CATAAAACCAACTTATTAATCCC	ACG
rs2278321	GCTCACAGGCTTTGTAACATC	ACT
rs2115874	GGGGAGATCTGCCATCTCCTGG	ACT
rs2033315	GGTAGTTGAGAGTATTGTGAGA	ACG
rs2033314	GTCTTGGTTTAAATCACTCCT	ACT
rs1991600	TAAAGGGGAAAAAAGCTCTAA	ACT
rs1864453	CTGCCAAGTTGAATACTGAGTT	ACT

Genetic Analysis

[0244] Allelotyping results from the discovery cohort are shown for cases and controls in Table 19. The allele frequency for the A2 allele is noted in the fifth and sixth columns for osteoarthritis case pools and control pools, respectively, where “AF” is allele frequency. The allele frequency for the A1 allele can be easily calculated by subtracting the A2 allele frequency from 1 (A1 AF = 1-A2 AF). For example, the SNP rs1971763 has the following case and control allele frequencies: case A1 (C) = 0.456; case A2 (T) = 0.544; control A1 (C) = 0.444; and control A2 (T) = 0.556, where the nucleotide is provided in paranthesis. Some SNPs are labeled “untyped” because of failed assays.

TABLE 19

dbSNP rs#	Position in SEQ ID NO: 3	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs1864455	209	175603909	C/T			
rs1971763	5908	175609608	C/T	0.544	0.556	0.630
rs934269	7460	175611160	A/G			
rs934270	7733	175611433	A/G			
rs2033309	7855	175611555	A/G	0.158	0.172	0.502
rs2033310	7904	175611604	A/C	0.428	0.423	0.845
rs934271	8869	175612569	G/T			
rs934272	9480	175613180	C/T			
rs1897110	13820	175617520	C/T			
rs2033311	15152	175618852	A/G			

dbSNP rs#	Position in SEQ ID NO: 3	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs1010027	17713	175621413	A/G			
rs1010028	17804	175621504	C/T	0.448	0.449	0.965
rs2884502	18220	175621920	C/T			
rs1430177	19083	175622783	C/T	0.051	0.309	~0.0001
rs1430178	19123	175622823	C/G			
rs3043779	19605	175623305	-/GTAAA			
rs1549742	20247	175623947	G/T			
rs3043781	20592	175624292	-/CCCCC			
rs2033313	21907	175625607	C/T			
rs7739	23273	175626973	C/T	0.057	0.042	0.371
rs11482	23299	175626999	A/C	0.934	0.935	0.958
rs3087907	23623	175627323	G/T	0.427	0.425	0.918
rs2358888	23669	175627369	A/T	0.083	0.064	0.245
rs1046036	23844	175627544	A/T			
rs3205060	24190	175627890	A/G	0.478	0.483	0.859
rs15327	24486	175628186	C/T	0.901	0.917	0.336
rs1430179	24896	175628596	A/C			
rs1430180	25118	175628818	C/G			
rs2163236	30551	175634251	C/G	0.956	0.955	0.994
rs3217351	30844	175634544	-/GAGA	0.481	0.487	0.823
rs2303891	30900	175634600	A/G	0.750	0.687	0.006
rs3815969	30942	175634642	A/G	0.232	0.239	0.771
rs2288622	31699	175635399	A/G	0.863	0.828	0.082
rs2288623	32081	175635781	G/T	0.081	0.106	0.134
rs1044335	35078	175638778	A/G	0.105	0.115	0.550
rs2288624	36196	175639896	A/T	0.901	0.871	0.117
rs1060511	36541	175640241	A/C	0.968	0.979	0.413
rs1367218	38356	175642056	A/G	0.931	0.958	0.068
rs1367217	45578	175649278	A/G	0.027	0.020	0.648
rs1465621	49634	175653334	A/T			
rs1465622	49774	175653474	G/T	0.084	0.108	0.161
rs2115872	51119	175654819	A/G	0.483	0.500	0.500
rs1465623	51181	175654881	A/G			
rs1469521	51652	175655352	C/T	0.433	0.435	0.953
rs1864451	54467	175658167	C/G	0.316	0.315	0.970
rs1430183	55762	175659462	A/G	0.972	0.970	0.930
rs1430182	55999	175659699	A/G	0.711	0.691	0.366
rs1430181	57865	175661565	A/C	0.939	0.943	0.836
rs1991601	66613	175670313	A/G	0.754	0.713	0.062
rs2358890	68377	175672077	C/T	0.404	0.443	0.109
rs2115875	69754	175673454	C/T	0.633	0.620	0.613
rs1430185	72859	175676559	A/G	0.768	0.750	0.445
rs2217429	76512	175680212	A/G	0.428	0.489	0.028
rs3049909	76717	175680417	-/AT	0.161	0.200	0.064
rs1430184	77722	175681422	C/T	0.025	untyped	NA
rs2278321	80998	175684698	A/G			
rs2115874	82033	175685733	C/T	0.729	0.698	0.179
rs2033315	89658	175693358	C/T	0.649	0.663	0.542
rs2033314	89960	175693660	A/G	0.697	0.692	0.835
rs1991600	94155	175697855	A/G	0.526	0.576	0.048
rs1864453	95679	175699379	A/G	0.675	0.672	0.883

[0245] The *WASPIP* proximal SNPs were also allelotyped in the replication cohorts using the methods described herein and the primers provided in Tables 17 and 18. The replication allelotyping results for replication cohort #1 and replication cohort #2 are provided in Tables 20 and 21, respectively.

TABLE 20

dbSNP rs#	Position in SEQ ID NO: 3	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs1864455	209	175603909	C/T			
rs1971763	5908	175609608	C/T	0.472	0.509	0.276
rs934269	7460	175611160	A/G			
rs934270	7733	175611433	A/G			
rs2033309	7855	175611555	A/G	0.179	0.186	0.784
rs2033310	7904	175611604	A/C	0.428	0.405	0.493
rs934271	8869	175612569	G/T			
rs934272	9480	175613180	C/T			
rs1897110	13820	175617520	C/T			
rs2033311	15152	175618852	A/G			
rs1010027	17713	175621413	A/G			
rs1010028	17804	175621504	C/T	0.447	0.465	0.579
rs2884502	18220	175621920	C/T			
rs1430177	19083	175622783	C/T	0.051	0.098	0.138
rs1430178	19123	175622823	C/G			
rs3043779	19605	175623305	-/GTAAA			
rs1549742	20247	175623947	G/T			
rs3043781	20592	175624292	-/CCCCC			
rs2033313	21907	175625607	C/T			
rs7739	23273	175626973	C/T	0.076	0.053	0.342
rs11482	23299	175626999	A/C	0.919	0.919	0.996
rs3087907	23623	175627323	G/T	0.422	0.390	0.348
rs2358888	23669	175627369	A/T	0.104	0.074	0.204
rs1046036	23844	175627544	A/T			
rs3205060	24190	175627890	A/G	0.501	0.472	0.391
rs15327	24486	175628186	C/T	0.883	0.904	0.370
rs1430179	24896	175628596	A/C			
rs1430180	25118	175628818	C/G			
rs2163236	30551	175634251	C/G	0.976	untyped	0.921
rs3217351	30844	175634544	-/GAGA	0.514	0.480	0.329
rs2303891	30900	175634600	A/G	0.780	0.699	0.009
rs3815969	30942	175634642	A/G	0.183	0.213	0.426
rs2288622	31699	175635399	A/G	0.856	0.818	0.201
rs2288623	32081	175635781	G/T	0.083	0.112	0.216
rs1044335	35078	175638778	A/G	0.113	0.115	0.959
rs2288624	36196	175639896	A/T	0.908	0.872	0.215
rs1060511	36541	175640241	A/C	0.971	untyped	0.945
rs1367218	38356	175642056	A/G	0.952	0.947	0.824
rs1367217	45578	175649278	A/G	0.020	untyped	NA
rs1465621	49634	175653334	A/T			
rs1465622	49774	175653474	G/T	0.077	0.118	0.108
rs2115872	51119	175654819	A/G	0.493	0.499	0.861
rs1465623	51181	175654881	A/G			
rs1469521	51652	175655352	C/T	0.453	0.427	0.436
rs1864451	54467	175658167	C/G	0.302	0.321	0.556
rs1430183	55762	175659462	A/G	0.959	0.962	0.903
rs1430182	55999	175659699	A/G	0.727	0.678	0.114

dbSNP rs#	Position in SEQ ID NO: 3	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs1430181	57865	175661565	A/C	0.942	0.940	0.943
rs1991601	66613	175670313	A/G	0.773	0.722	0.081
rs2358890	68377	175672077	C/T	0.389	0.443	0.111
rs2115875	69754	175673454	C/T	0.639	0.601	0.267
rs1430185	72859	175676559	A/G	0.790	0.774	0.586
rs2217429	76512	175680212	A/G	0.412	0.504	0.029
rs3049909	76717	175680417	-/AT	0.144	0.193	0.079
rs1430184	77722	175681422	C/T			
rs2278321	80998	175684698	A/G			
rs2115874	82033	175685733	C/T	0.744	0.703	0.169
rs2033315	89658	175693358	C/T	0.675	0.695	0.533
rs2033314	89960	175693660	A/G	0.726	0.703	0.529
rs1991600	94155	175697855	A/G	0.467	0.566	0.005
rs1864453	95679	175699379	A/G	0.702	0.680	0.468

TABLE 21

dbSNP rs#	Position in SEQ ID NO: 3	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs1864455	209	175603909	C/T			
rs1971763	5908	175609608	C/T	0.635	0.629	0.879
rs934269	7460	175611160	A/G			
rs934270	7733	175611433	A/G			
rs2033309	7855	175611555	A/G	0.131	0.149	0.576
rs2033310	7904	175611604	A/C	0.428	0.452	0.548
rs934271	8869	175612569	G/T			
rs934272	9480	175613180	C/T			
rs1897110	13820	175617520	C/T			
rs2033311	15152	175618852	A/G			
rs1010027	17713	175621413	A/G			
rs1010028	17804	175621504	C/T	0.449	0.424	0.503
rs2884502	18220	175621920	C/T			
rs1430177	19083	175622783	C/T	untyped	0.642	NA
rs1430178	19123	175622823	C/G			
rs3043779	19605	175623305	-/GTAAA			
rs1549742	20247	175623947	G/T			
rs3043781	20592	175624292	-/CCCCC			
rs2033313	21907	175625607	C/T			
rs7739	23273	175626973	C/T	0.032	0.023	0.700
rs11482	23299	175626999	A/C	0.953	0.960	0.743
rs3087907	23623	175627323	G/T	0.435	0.479	0.295
rs2358888	23669	175627369	A/T	0.055	0.048	0.731
rs1046036	23844	175627544	A/T			
rs3205060	24190	175627890	A/G	0.449	0.500	0.197
rs15327	24486	175628186	C/T	0.923	0.937	0.552
rs1430179	24896	175628596	A/C			
rs1430180	25118	175628818	C/G			
rs2163236	30551	175634251	C/G	0.923	untyped	
rs3217351	30844	175634544	-/GAGA	0.439	0.496	0.125
rs2303891	30900	175634600	A/G	0.712	0.667	0.208
rs3815969	30942	175634642	A/G	0.294	0.281	0.705
rs2288622	31699	175635399	A/G	0.872	0.843	0.309
rs2288623	32081	175635781	G/T	0.078	0.096	0.444

dbSNP rs#	Position in SEQ ID NO: 3	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs1044335	35078	175638778	A/G	0.094	0.117	0.366
rs2288624	36196	175639896	A/T	0.894	0.869	0.356
rs1060511	36541	175640241	A/C			
rs1367218	38356	175642056	A/G	0.903	0.976	0.001
rs1367217	45578	175649278	A/G	0.035	0.021	0.504
rs1465621	49634	175653334	A/T			
rs1465622	49774	175653474	G/T	0.092	0.093	0.959
rs2115872	51119	175654819	A/G	0.471	0.502	0.397
rs1465623	51181	175654881	A/G			
rs1469521	51652	175655352	C/T	0.408	0.447	0.282
rs1864451	54467	175658167	C/G	0.334	0.306	0.420
rs1430183	55762	175659462	A/G			
rs1430182	55999	175659699	A/G	0.691	0.711	0.547
rs1430181	57865	175661565	A/C	0.936	0.946	0.651
rs1991601	66613	175670313	A/G	0.730	0.700	0.372
rs2358890	68377	175672077	C/T	0.423	0.444	0.566
rs2115875	69754	175673454	C/T	0.625	0.650	0.510
rs1430185	72859	175676559	A/G	0.740	0.714	0.462
rs2217429	76512	175680212	A/G	0.447	0.464	0.644
rs3049909	76717	175680417	-/AT	0.184	0.212	0.386
rs1430184	77722	175681422	C/T			
rs2278321	80998	175684698	A/G			
rs2115874	82033	175685733	C/T	0.709	0.691	0.605
rs2033315	89658	175693358	C/T	0.616	0.614	0.962
rs2033314	89960	175693660	A/G	0.660	0.674	0.679
rs1991600	94155	175697855	A/G	0.602	0.590	0.749
rs1864453	95679	175699379	A/G	0.641	0.659	0.631

[0246] Allelotyping results were considered particularly significant with a calculated p-value of less than or equal to 0.05 for allelotype results. These values are indicated in bold. The allelotyping p-values were plotted in Figure 1C for the discovery cohort. The position of each SNP on the chromosome is presented on the x-axis. The y-axis gives the negative logarithm (base 10) of the p-value comparing the estimated allele in the case group to that of the control group. The minor allele frequency of the control group for each SNP designated by an X or other symbol on the graphs in Figure 1C can be determined by consulting Table 19. For example, the left-most X on the left graph is at position 175603909. By proceeding down the Table from top to bottom and across the graphs from left to right the allele frequency associated with each symbol shown can be determined.

[0247] To aid the interpretation, multiple lines have been added to the graph. The broken horizontal lines are drawn at two common significance levels, 0.05 and 0.01. The vertical broken lines are drawn every 20kb to assist in the interpretation of distances between SNPs. Two other lines are drawn to expose linear trends in the association of SNPs to the disease. The light gray line (or generally bottom-most curve) is a nonlinear smoother through the data points on the graph using a local polynomial regression method (W.S. Cleveland, E. Grosse and W.M. Shyu (1992) Local regression models. Chapter

8 of Statistical Models in S eds J.M. Chambers and T.J. Hastie, Wadsworth & Brooks/Cole.). The black line provides a local test for excess statistical significance to identify regions of association. This was created by use of a 10kb sliding window with 1kb step sizes. Within each window, a chi-square goodness of fit test was applied to compare the proportion of SNPs that were significant at a test wise level of 0.01, to the proportion that would be expected by chance alone (0.05 for the methods used here). Resulting p-values that were less than 10^{-8} were truncated at that value.

[0248] Finally, the exons and introns of the genes in the covered region are plotted below each graph at the appropriate chromosomal positions. The gene boundary is indicated by the broken horizontal line. The exon positions are shown as thick, unbroken bars. An arrow is place at the 3' end of each gene to show the direction of transcription.

Example 6

LOXL1 Region Proximal SNPs

[0249] It has been discovered that rs8818 in the untranslated region (UTR) of the lysyl oxidase-like 1 (LOXL1) gene is associated with occurrence of osteoarthritis in subjects. LOXL1 is a Lysyl oxidase-like protein that catalyzes the cross-linking of collagen via lysine residues. Deficiency of the related protein, lysyl oxidase, causes a form of Ehlers-Danlos syndrome. LOXL1 likely is a secreted protein and its biological activity may be modulated by addition of an antibody, a recombinant binding partner, a binding agent, or a recombinant LOXL1 protein or functional fragment thereof.

[0250] Fifty-eight additional allelic variants proximal to rs912428 were identified and subsequently allelotyped in osteoarthritis case and control sample sets as described in Examples 1 and 2. The polymorphic variants are set forth in Table 22. The chromosome positions provided in column four of Table 22 are based on Genome “Build 34” of NCBI’s GenBank.

TABLE 22

dbSNP rs#	Chromosome	Position in SEQ ID NO: 5	Chromosome Position	Allele Variants
rs1048661	15	213	71935363	G/T
rs3825942	15	249	71935399	C/T
rs1550436	15	1824	71936974	C/T
rs1550438	15	2057	71937207	C/T
rs1550439	15	2306	71937456	A/T
rs2165241	15	2869	71938019	C/T
rs1550433	15	3976	71939126	A/C
rs3056314	15	4288	71939438	-/TC
rs2415204	15	4290	71939440	A/C
rs1992314	15	4434	71939584	C/G

dbSNP rs#	Chromosome	Position in SEQ ID NO: 5	Chromosome Position	Allele Variants
rs1440101	15	5298	71940448	A/G
rs2289414	15	5467	71940617	A/G
rs2415205	15	8486	71943636	C/G
rs2899807	15	8487	71943637	A/T
rs893815	15	8831	71943981	C/G
rs3056342	15	9036	71944186	-/AG
rs4077284	15	9058	71944208	A/G
rs893816	15	9131	71944281	C/T
rs893817	15	9732	71944882	A/G
rs893818	15	9862	71945012	A/G
rs893819	15	10191	71945341	A/G
rs893820	15	10270	71945420	C/T
rs2304719	15	16167	71951317	C/T
rs1001507	15	17620	71952770	G/T
rs1530167	15	17751	71952901	C/T
rs1530168	15	17764	71952914	C/T
rs1530169	15	17787	71952937	C/T
rs2304720	15	19401	71954551	C/T
rs2304721	15	21021	71956171	A/C
rs893821	15	21902	71957052	C/T
rs750460	15	22173	71957323	C/T
rs2304722	15	22416	71957566	C/T
rs1440102	15	22653	71957803	A/G
rs8818	15	24945	71960095	C/G
rs3522	15	25011	71960161	C/T
rs2415206	15	28563	71963713	C/T
rs1984526	15	48574	71983724	C/G
rs1984525	15	48710	71983860	C/T
rs3031653	15	48880	71984030	-/TTG
rs2415187	15	50194	71985344	C/T
rs2507	15	56343	71991493	A/G
rs2289411	15	56455	71991605	C/T
rs3202077	15	56729	71991879	C/T
rs2289412	15	56759	71991909	A/G
rs2289413	15	56895	71992045	A/G
rs1061082	15	57036	71992186	C/G
rs2277600	15	57702	71992852	C/G
rs734854	15	62515	71997665	C/T
rs2415188	15	62629	71997779	C/G
rs3214695	15	63501	71998651	-/C
rs3816197	15	63547	71998697	C/T
rs3816198	15	64876	72000026	C/G
rs2304715	15	65073	72000223	C/G
rs2301272	15	67149	72002299	C/T
rs2301273	15	67549	72002699	C/T
rs3784563	15	71660	72006810	A/C

dbSNP rs#	Chromosome	Position in SEQ ID NO: 5	Chromosome Position	Allele Variants
rs3784561	15	71906	72007056	C/T
rs3784560	15	71911	72007061	A/C

Assay for Verifying and Allelotyping SNPs

[0251] The methods used to verify and allelotype the 58 proximal SNPs of Table 22 are the same methods described in Examples 1 and 2 herein. The primers and probes used in these assays are provided in Table 23 and Table 24, respectively.

TABLE 23

dbSNP rs#	Forward PCR primer	Reverse PCR primer
rs1048661	ACGTTGGATGTTGCTGGGAGACGGAGGTG	ACGTTGGATGATTCCGGCTTTGGCCAGGTGC
rs3825942	ACGTTGGATGTAGGTGCTGGCGAAGGCCGAA	ACGTTGGATGACCTCCGTCTCCCAGCAAC
rs1130133	ACGTTGGATGACCAAGTCAGGGAGACCGCG	ACGTTGGATGAGCGGAACGGCGCGCAGCA
rs1550436	ACGTTGGATGGCCAAAAAACTCAGTAACG	ACGTTGGATGGTTCATTACAGATAGTTTGC
rs1550437	ACGTTGGATGTTGGGCCTTCCCAAGAGGAG	ACGTTGGATGAGAGCCCCAGCTGTGGACA
rs1550438	ACGTTGGATGAGTCAGCCCTTGTACAGTA	ACGTTGGATGCATGAGGACACAGTGGAAAG
rs1550439	ACGTTGGATGATTCTCTGCTCCCCATTGAG	ACGTTGGATGTATACTCTGAGGCACTGGAG
rs2165241	ACGTTGGATGTAGAAGACCCACTGACTTGG	ACGTTGGATGGGGCAGAGAAAACCTGAGCTC
rs1550433	ACGTTGGATGATAGCAGGAGTGGTCACATC	ACGTTGGATGTAGCAAATCCTTGAAGAGAG
rs3056314	ACGTTGGATGTCTCTCCTGGCCTCTGATTG	ACGTTGGATGCCTGACGTGTGTCTCTATC
rs2415204	ACGTTGGATGGTTCTCTCCTGGCCTCTGAT	ACGTTGGATGCCTGACGTGTGTCTCTATC
rs1992314	ACGTTGGATGTTTGTCTAAAGGCCCTGAG	ACGTTGGATGAGATAAACCCCTGCAGTCTG
rs1440101	ACGTTGGATGAAAAGTCAGCAAGTGAGCTC	ACGTTGGATGTTAATTCCCAGGTCCTAGCC
rs2289414	ACGTTGGATGTTGCTTATCTTGACACCTC	ACGTTGGATGCTCACCCCTGTCACAACCACT
rs2415205	ACGTTGGATGTGATGCTTCAGTTACTCCAG	ACGTTGGATGTGTGGGCAGCGTAAGTTTTC
rs2899807	ACGTTGGATGTGATGCTTCAGTTACTCCAG	ACGTTGGATGTGTGGGCAGCGTAAGTTTTC
rs893815	ACGTTGGATGCACCCTTTACAGCACTCAC	ACGTTGGATGATCCCTTCTGTGAGTCAAGC
rs3056342	ACGTTGGATGTAAGGATCAGTAGGCAGGTC	ACGTTGGATGATAGCTGGGAATCCAGGAC
rs4077284	ACGTTGGATGTAAGGATCAGTAGGCAGGTC	ACGTTGGATGATAGCTGGGAATCCAGGAC
rs893816	ACGTTGGATGATTGCCACAGAATCAAGCC	ACGTTGGATGTTCTGGAAGGCTAGGTAAGG
rs893817	ACGTTGGATGAAACAGGTGAGGTGTGGACG	ACGTTGGATGAGAAATCTGTTCCCTCCTGC
rs893818	ACGTTGGATGTTTTAGGAGCTGTTCAAGTC	ACGTTGGATGTGGGAGAATTTCTGACTGC
rs893819	ACGTTGGATGCTGTACACTGACTCTTGGG	ACGTTGGATGATGGTCTTTGTCTCCGGTT
rs893820	ACGTTGGATGAGAGTCAGTGTGACAGGTTT	ACGTTGGATGTTCTATATCTGCTGCTGCC
rs2304719	ACGTTGGATGTTTCATCAGTGAGCCTTGCC	ACGTTGGATGCCTTGATAGTGAGGTACAG
rs1001507	ACGTTGGATGAGAATCCTGCAAACTGGAG	ACGTTGGATGTGCAGCATGTGAAGTGGCAC
rs1530167	ACGTTGGATGAAACATCCTCCTTCCCTCTG	ACGTTGGATGGCCTAGAACCTAGACCCTTA
rs1530168	ACGTTGGATGCAAAACATCCTCCTTCCCTC	ACGTTGGATGGACCTTATGGTTTCCCATG
rs1530169	ACGTTGGATGTGTGCTGAGCTGAACAGAAG	ACGTTGGATGGGAATCTGTCTATGTCTGGG
rs2304720	ACGTTGGATGATGCTGGGTTCTGGTGTCAC	ACGTTGGATGATAGGCTGTGCTGCAGGGAC

dbSNP rs#	Forward PCR primer	Reverse PCR primer
rs2304721	ACGTTGGATGCTCAAGTGATGCCTCAGATG	ACGTTGGATGCTGAAAGAAGCTTCAGCCTC
rs893821	ACGTTGGATGTGGATTAAGTAGGGTAGGGC	ACGTTGGATGGAAAGTTGCATCCCTGCATC
rs750460	ACGTTGGATGATGTTCCCTAGAGCTAGAG	ACGTTGGATGCTCAGCTCCTCATTACTGCA
rs2304722	ACGTTGGATGTTACCACCTTCTCTGGTGAG	ACGTTGGATGGAGGAAGAAGAGAAACAGGG
rs1440102	ACGTTGGATGAGTAAGAGTTTCCCCACCAC	ACGTTGGATGTGACCTAAAGTGCAGGTATC
rs8818	ACGTTGGATGAATCTCTCCCTTCCAAAGC	ACGTTGGATGTCCCTGTGGTTTTTCATCCAC
rs3522	ACGTTGGATGAACAACACTGTAGAGAAAAGTGAA	ACGTTGGATGACGTGGATGAAAACCACAGG
rs2415206	ACGTTGGATGCACCTTGAGGTGAAACAGAC	ACGTTGGATGTTACTTAGTAGACCCCGAGG
rs1984526	ACGTTGGATGATCCTTTGTTCTTGAAACAG	ACGTTGGATGGGATTACAAACGTGAGCCAC
rs1984525	ACGTTGGATGCAGCTGGGATTACAGGTATG	ACGTTGGATGACCAACATGGTGAAACCCTG
rs3031653	ACGTTGGATGATAAACGTTAAGCTCAGTTG	ACGTTGGATGAAAAAAGAGTGAAGTCG
rs2415187	ACGTTGGATGTTCTATGAGTTACTTGACAC	ACGTTGGATGGTGCTTTATCTGACTAGTG
rs2507	ACGTTGGATGGCTGCTCCCAAAGATTTCTG	ACGTTGGATGTAAGAAGCACAGAACGCAGG
rs2289411	ACGTTGGATGCTGTGGCGAAGTTACCTGGG	ACGTTGGATGTGCTCCTTCCCATGCCAAT
rs3202077	ACGTTGGATGACAGTGTTCTCTGGACAAG	ACGTTGGATGTCTCCTCCTGGAATCACACC
rs2289412	ACGTTGGATGGGACAAAGCCTTGTCCAGAG	ACGTTGGATGATGAATGGAGGCTGCAGGAG
rs2289413	ACGTTGGATGTTGGCTGACTTTCCAGAGCC	ACGTTGGATGTGCAGATGAACACCTCCTCC
rs1061082	ACGTTGGATGGGCCCTGCTATGCAGAGAG	ACGTTGGATGAGGTCGCCCTTACCTTCAG
rs2277600	ACGTTGGATGTAGTGAGGTCCAGGAAGTAG	ACGTTGGATGCCTGCTACCAGTTCAATGTC
rs734854	ACGTTGGATGATAACTCCAAAGGCCATGTG	ACGTTGGATGCAGACCACAGAGATGAAAAG
rs2415188	ACGTTGGATGAAAGTTGACAAAGCCCTTTC	ACGTTGGATGAGGAAACTGTCTGTCTTGG
rs3214695	ACGTTGGATGACACTTGCCCCAAGTTCACTC	ACGTTGGATGTACATCTGCAGGTGAGAGCA
rs3816197	ACGTTGGATGGTGAACCTGGGCAAGTGATC	ACGTTGGATGAGATTGAGAGCCCTGAGAAG
rs3816198	ACGTTGGATGTAGGGTCATGGGGCTTTGG	ACGTTGGATGGGCTGATAAGAGCCGAGGAC
rs2304715	ACGTTGGATGGTGAGTGGCCGCCTGGCAC	ACGTTGGATGTCCTCGGAGGCAGAGATTG
rs2301272	ACGTTGGATGATGATACCCAAGGAGTGTGC	ACGTTGGATGTCAGCAACTTCCCATCACTC
rs2301273	ACGTTGGATGACCTACCGCTGACTTACGG	ACGTTGGATGACGGATGAATGGATCAAAG
rs3784563	ACGTTGGATGAATGTGGTCTGCAGATATGC	ACGTTGGATGAACTTACTATCCACCTGCG
rs3784561	ACGTTGGATGATGACCACAATTTATGCTGC	ACGTTGGATGTGCAAAGATGATTCTGCAGC
rs3784561	ACGTTGGATGCAGTAAGGCTGGATTCTAGG	ACGTTGGATGGCTGCCTGGTGTAAATGGTT
rs3784560	ACGTTGGATGGCTGGATTCTAGGATCAGAG	ACGTTGGATGACATTCTCAGATAGCGCTGC

TABLE 24

dbSNP rs#	Extend Primer	Term Mix
rs1048661	GGAGACGGAGGTGCGGGCC	CGT
rs3825942	GAGACCGAGGAGGCGGAG	ACG
rs1130133	GGCCGGTACACGCTGCC	ACG
rs1550436	AAAAAACTCAGTAACGGAGATAA	ACT
rs1550437	TTCACCCCTGAAAAGCCAGA	ACT
rs1550438	GTAGCCCTGTCTGCTAACAGCAT	ACT
rs1550439	CTCCCCATTGAGGTTGCTG	CGT
rs2165241	CCAGGCATGCCTCTGCCA	ACT

dbSNP rs#	Extend Primer	Term Mix
rs1550433	GGTCACATCGAGGGAGCC	ACT
rs3056314	TGGCCTCTGATTGGCCATG	ACT
rs2415204	CTCCTGGCCTCTGATTGGCCA	ACT
rs1992314	AAGGCCCTGAGGAGCTACA	ACT
rs1440101	CTCGTCACCACATCTGTAACA	ACG
rs2289414	TTTATTCACTCATTCTTTGGTC	ACT
rs2415205	CTCAGGCCCTGCACAGTGA	ACT
rs2899807	CTCAGGCCCTGCACAGTG	CGT
rs893815	ACAGCACTCACCTGTCCAC	ACT
rs3056342	CACACCCCAACCTTTTTTACCCC	ACT
rs4077284	GGCAGGTCTCTGGCAGCA	ACG
rs893816	CAGAGTGGCAGCTAAAGCC	ACT
rs893817	GGTGTGGACGAGCAATGGGAA	ACT
rs893818	AGCCCTCTCACAACCCCTACAGA	ACG
rs893819	CACCTGTCTCTCTGCTCAA	ACG
rs893820	ACAGGTTCTCTCTACTGTGC	ACG
rs2304719	CAGGAGGGGAGGGGAGCAAG	ACG
rs1001507	GGCCCTCTGAGATCATTTCAA	ACT
rs1530167	CTGTTCTAGCTCAGCACACC	ACT
rs1530168	CAGTTAAATCCTGCCCTTCTGTTT	ACT
rs1530169	TGAACAGAAGGGCAGGATTTAAC	ACG
rs2304720	TGTGCCCCAACCCCCCCC	ACG
rs2304721	TCAGATGCTGCCTCTGCTC	ACT
rs893821	GCCAGCTTTATTTGCAGAACATCT	ACT
rs750460	CAGAGAGGTTGGATCCTGCC	ACG
rs2304722	CTCTGGTGAGCAGTTGAGG	ACG
rs1440102	GCAGGCAAGGCCACCTGA	ACT
rs8818	AGCCCCCAACCCACAGGCA	ACT
rs3522	TATAAAATGGGGTCTGGC	ACT
rs2415206	GAAACAGACCCCCACCCC	ACG
rs1984526	AGCATAAAGGTGAAAGATGGGCC	ACT
rs1984525	GGATTACAGGTATGCACCACCA	ACG
rs3031653	AAGCTCAGTTGTGGCTCCAAACAA	ACT
rs2415187	TCTTTTTAAAAAACTACACCAGGT	ACG
rs2507	TGACTCATCTGCCAGCTC	ACG
rs2289411	GGGATCCTGGCTGGCCC	ACT
rs3202077	CTGGACAAGGCTTTGTCCAT	ACG
rs2289412	GCCTTGTCCAGAGAACCACT	ACT
rs2289413	CAAGCCTGGCACCAAGCC	ACG
rs1061082	CTATGCAGAGAGCTGCGGC	ACT
rs2277600	GGAAGTAGGCGCTTTGGGTG	ACT
rs734854	ACTCCAAAGGCCATGTGTCTTAAC	ACG
rs2415188	GGGGTGCTGTTAGGGCAGCC	ACT

dbSNP rs#	Extend Primer	Term Mix
rs3214695	CGCTTGGCAGCTGTCGTG	ACT
rs3816197	CTTGGGCAAGTGTACCTTACG	ACG
rs3816198	CCCCAGAGCCAGCCAGC	ACT
rs2304715	CCGCCTGGCACGGCGGA	ACT
rs2301272	TGTGCTAGGACAAGATCCTAGCT	ACT
rs2301273	GCTGACTTACGGTAAAGCGG	ACT
rs3784563	TGACCACAATTTATGCTGCCA	ACT
rs3784561	GCAGGTGGATAGTAAGTTTCCA	ACT
rs3784561	GCTGGATTCTAGGATCAGAGACA	ACT
rs3784560	CTAGGATCAGAGACAGGTAG	ACT

Genetic Analysis

[0252] Allelotyping results from the discovery cohort are shown for cases and controls in Table 25. The allele frequency for the A2 allele is noted in the fifth and sixth columns for osteoarthritis case pools and control pools, respectively, where “AF” is allele frequency. The allele frequency for the A1 allele can be easily calculated by subtracting the A2 allele frequency from 1 (A1 AF = 1-A2 AF). For example, the SNP rs1048661 has the following case and control allele frequencies: case A1 (G) = 0.725; case A2 (T) = 0.275; control A1 (G) = 0.767; and control A2 (T) = 0.233, where the nucleotide is provided in paranthesis. Some SNPs are labeled “untyped” because of failed assays.

TABLE 25

dbSNP rs#	Position in SEQ ID NO: 5	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs1048661	213	71935363	G/T	0.275	0.233	0.077
rs3825942	249	71935399	C/T	0.107	0.148	0.056
rs1550436	1824	71936974	C/T	0.401	0.420	0.470
rs1550438	2057	71937207	C/T			
rs1550439	2306	71937456	A/T			
rs2165241	2869	71938019	C/T	0.427	0.430	0.883
rs1550433	3976	71939126	A/C			
rs3056314	4288	71939438	-/TC			
rs2415204	4290	71939440	A/C	0.176	0.177	0.982
rs1992314	4434	71939584	C/G	0.599	0.601	0.938
rs1440101	5298	71940448	A/G			
rs2289414	5467	71940617	A/G			
rs2415205	8486	71943636	C/G			
rs2899807	8487	71943637	A/T	0.951	0.956	0.863
rs893815	8831	71943981	C/G			
rs3056342	9036	71944186	-/AG	0.290	0.292	0.927
rs4077284	9058	71944208	A/G	0.358	0.358	0.985
rs893816	9131	71944281	C/T	0.517	0.515	0.928
rs893817	9732	71944882	A/G	0.162	0.158	0.819
rs893818	9862	71945012	A/G	0.311	0.313	0.920

dbSNP rs#	Position in SEQ ID NO: 5	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs893819	10191	71945341	A/G	0.637	0.642	0.866
rs893820	10270	71945420	C/T	0.901	0.910	0.605
rs2304719	16167	71951317	C/T	0.320	0.299	0.387
rs1001507	17620	71952770	G/T	0.910	0.916	0.709
rs1530167	17751	71952901	C/T			
rs1530168	17764	71952914	C/T			
rs1530169	17787	71952937	C/T	0.209	0.203	0.779
rs2304720	19401	71954551	C/T	0.942	0.947	0.724
rs2304721	21021	71956171	A/C	0.798	0.814	0.519
rs893821	21902	71957052	C/T	0.113	0.116	0.879
rs750460	22173	71957323	C/T	0.473	0.438	0.176
rs2304722	22416	71957566	C/T	0.744	0.747	0.926
rs1440102	22653	71957803	A/G			
rs8818	24945	71960095	C/G			
rs3522	25011	71960161	C/T	0.424	0.441	0.472
rs2415206	28563	71963713	C/T	0.376	0.366	0.731
rs1984526	48574	71983724	C/G	0.593	untyped	NA
rs1984525	48710	71983860	C/T			
rs3031653	48880	71984030	-/TTG			
rs2415187	50194	71985344	C/T			
rs2507	56343	71991493	A/G	0.655	0.653	0.924
rs2289411	56455	71991605	C/T			
rs3202077	56729	71991879	C/T			
rs2289412	56759	71991909	A/G	0.971	0.968	0.855
rs2289413	56895	71992045	A/G	0.972	0.972	0.997
rs1061082	57036	71992186	C/G			
rs2277600	57702	71992852	C/G			
rs734854	62515	71997665	C/T	0.381	0.379	0.915
rs2415188	62629	71997779	C/G	0.532	0.538	0.832
rs3214695	63501	71998651	-/C	0.308	0.300	0.751
rs3816197	63547	71998697	C/T	0.327	0.311	0.512
rs3816198	64876	72000026	C/G	0.598	0.584	0.575
rs2304715	65073	72000223	C/G	0.660	0.643	0.534
rs2301272	67149	72002299	C/T	0.974	0.972	0.853
rs2301273	67549	72002699	C/T	0.952	0.966	0.409
rs3784563	71660	72006810	A/C	0.495	0.508	0.590
rs3784561	71906	72007056	C/T	0.470	0.466	0.872
rs3784560	71911	72007061	A/C			

[0253] The *LOXLI* proximal SNPs were also allelotyped in the replication cohorts using the methods described herein and the primers provided in Tables 23 and 24. The replication allelotyping results for replication cohort #1 and replication cohort #2 are provided in Tables 26 and 27, respectively.

TABLE 26

dbSNP rs#	Position in SEQ ID NO: 5	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs1048661	213	71935363	G/T	0.250	0.252	0.953
rs3825942	249	71935399	C/T	0.126	0.141	0.539
rs1550436	1824	71936974	C/T	0.397	0.405	0.845

dbSNP rs#	Position in SEQ ID NO: 5	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs1550438	2057	71937207	C/T			
rs1550439	2306	71937456	A/T			
rs2165241	2869	71938019	C/T	0.429	0.425	0.894
rs1550433	3976	71939126	A/C			
rs3056314	4288	71939438	-/TC			
rs2415204	4290	71939440	A/C	0.162	untyped	0.176
rs1992314	4434	71939584	C/G	0.583	0.594	0.756
rs1440101	5298	71940448	A/G			
rs2289414	5467	71940617	A/G			
rs2415205	8486	71943636	C/G			
rs2899807	8487	71943637	A/T	0.939	untyped	NA
rs893815	8831	71943981	C/G			
rs3056342	9036	71944186	-/AG	0.317	0.311	0.846
rs4077284	9058	71944208	A/G	0.372	0.365	0.881
rs893816	9131	71944281	C/T	0.510	0.518	0.793
rs893817	9732	71944882	A/G	0.178	0.170	0.784
rs893818	9862	71945012	A/G	0.327	0.320	0.818
rs893819	10191	71945341	A/G	0.610	untyped	NA
rs893820	10270	71945420	C/T	0.874	0.903	0.218
rs2304719	16167	71951317	C/T	0.309	0.289	0.537
rs1001507	17620	71952770	G/T	0.908	0.924	0.525
rs1530167	17751	71952901	C/T			
rs1530168	17764	71952914	C/T			
rs1530169	17787	71952937	C/T	0.237	0.202	0.249
rs2304720	19401	71954551	C/T	0.935	0.944	0.661
rs2304721	21021	71956171	A/C	0.759	0.823	0.091
rs893821	21902	71957052	C/T	0.114	0.122	0.778
rs750460	22173	71957323	C/T	0.469	0.440	0.433
rs2304722	22416	71957566	C/T	0.729	0.746	0.572
rs1440102	22653	71957803	A/G			
rs8818	24945	71960095	C/G			
rs3522	25011	71960161	C/T	0.416	0.440	0.454
rs2415206	28563	71963713	C/T	0.362	untyped	NA
rs1984526	48574	71983724	C/G	0.593	untyped	
rs1984525	48710	71983860	C/T			
rs3031653	48880	71984030	-/TTG			
rs2415187	50194	71985344	C/T			
rs2507	56343	71991493	A/G	0.676	0.653	0.471
rs2289411	56455	71991605	C/T			
rs3202077	56729	71991879	C/T			
rs2289412	56759	71991909	A/G	0.964	0.954	0.626
rs2289413	56895	71992045	A/G	0.963	0.959	0.833
rs1061082	57036	71992186	C/G			
rs2277600	57702	71992852	C/G			
rs734854	62515	71997665	C/T	0.403	0.383	0.531
rs2415188	62629	71997779	C/G	0.555	0.564	0.809
rs3214695	63501	71998651	-/C	0.289	0.300	0.721
rs3816197	63547	71998697	C/T	0.304	0.308	0.904
rs3816198	64876	72000026	C/G	0.601	0.598	0.922
rs2304715	65073	72000223	C/G	0.649	0.678	0.457
rs2301272	67149	72002299	C/T	0.966	0.959	0.752
rs2301273	67549	72002699	C/T	0.935	0.946	0.649
rs3784563	71660	72006810	A/C	0.502	0.516	0.685
rs3784561	71906	72007056	C/T	0.438	0.471	0.319
rs3784560	71911	72007061	A/C			

TABLE 27

dbSNP rs#	Position in SEQ ID NO: 5	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs1048661	213	71935363	G/T	0.307	0.203	0.007
rs3825942	249	71935399	C/T	0.084	0.159	0.031
rs1550436	1824	71936974	C/T	0.406	0.445	0.274
rs1550438	2057	71937207	C/T			
rs1550439	2306	71937456	A/T			
rs2165241	2869	71938019	C/T	0.423	0.439	0.669
rs1550433	3976	71939126	A/C			
rs3056314	4288	71939438	-/TC			
rs2415204	4290	71939440	A/C	0.200	untyped	
rs1992314	4434	71939584	C/G	0.618	0.612	0.854
rs1440101	5298	71940448	A/G			
rs2289414	5467	71940617	A/G			
rs2415205	8486	71943636	C/G			
rs2899807	8487	71943637	A/T	0.965	0.956	0.737
rs893815	8831	71943981	C/G			
rs3056342	9036	71944186	-/AG	0.257	0.264	0.833
rs4077284	9058	71944208	A/G	0.341	0.345	0.905
rs893816	9131	71944281	C/T	0.526	0.509	0.655
rs893817	9732	71944882	A/G	0.142	0.139	0.895
rs893818	9862	71945012	A/G	0.290	0.302	0.712
rs893819	10191	71945341	A/G	0.671	0.642	0.431
rs893820	10270	71945420	C/T	0.934	0.922	0.681
rs2304719	16167	71951317	C/T	0.334	0.316	0.613
rs1001507	17620	71952770	G/T	0.911	0.903	0.741
rs1530167	17751	71952901	C/T			
rs1530168	17764	71952914	C/T			
rs1530169	17787	71952937	C/T	0.173	0.203	0.360
rs2304720	19401	71954551	C/T	0.951	0.952	0.952
rs2304721	21021	71956171	A/C	0.848	0.799	0.150
rs893821	21902	71957052	C/T	0.112	0.106	0.829
rs750460	22173	71957323	C/T	0.478	0.435	0.242
rs2304722	22416	71957566	C/T	0.764	0.748	0.626
rs1440102	22653	71957803	A/G			
rs8818	24945	71960095	C/G			
rs3522	25011	71960161	C/T	0.435	0.444	0.814
rs2415206	28563	71963713	C/T	0.394	0.366	0.419
rs1984526	48574	71983724	C/G			
rs1984525	48710	71983860	C/T			
rs3031653	48880	71984030	-/TTG			
rs2415187	50194	71985344	C/T			
rs2507	56343	71991493	A/G	0.630	0.653	0.509
rs2289411	56455	71991605	C/T			
rs3202077	56729	71991879	C/T			
rs2289412	56759	71991909	A/G	0.979	untyped	
rs2289413	56895	71992045	A/G			
rs1061082	57036	71992186	C/G			
rs2277600	57702	71992852	C/G			
rs734854	62515	71997665	C/T	0.354	0.372	0.611
rs2415188	62629	71997779	C/G	0.502	0.497	0.897
rs3214695	63501	71998651	-/C	0.331	0.300	0.367
rs3816197	63547	71998697	C/T	0.357	0.317	0.259
rs3816198	64876	72000026	C/G	0.594	0.562	0.416
rs2304715	65073	72000223	C/G	0.674	0.587	0.020
rs2301272	67149	72002299	C/T			

dbSNP rs#	Position in SEQ ID NO: 5	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs2301273	67549	72002699	C/T	0.973	untyped	
rs3784563	71660	72006810	A/C	0.485	0.496	0.777
rs3784561	71906	72007056	C/T	0.511	0.459	0.174
rs3784560	71911	72007061	A/C			

[0254] Allelotyping results were considered particularly significant with a calculated p-value of less than or equal to 0.05 for allelotype results. These values are indicated in bold. The allelotyping p-values were plotted in Figure 1E for the discovery cohort. The position of each SNP on the chromosome is presented on the x-axis. The y-axis gives the negative logarithm (base 10) of the p-value comparing the estimated allele in the case group to that of the control group. The minor allele frequency of the control group for each SNP designated by an X or other symbol on the graphs in Figure 1E can be determined by consulting Table 25. For example, the left-most X on the left graph is at position 71935363. By proceeding down the Table from top to bottom and across the graphs from left to right the allele frequency associated with each symbol shown can be determined.

[0255] To aid the interpretation, multiple lines have been added to the graph. The broken horizontal lines are drawn at two common significance levels, 0.05 and 0.01. The vertical broken lines are drawn every 20kb to assist in the interpretation of distances between SNPs. Two other lines are drawn to expose linear trends in the association of SNPs to the disease. The light gray line (or generally bottom-most curve) is a nonlinear smoother through the data points on the graph using a local polynomial regression method (W.S. Cleveland, E. Grosse and W.M. Shyu (1992) Local regression models. Chapter 8 of Statistical Models in S eds J.M. Chambers and T.J. Hastie, Wadsworth & Brooks/Cole.). The black line provides a local test for excess statistical significance to identify regions of association. This was created by use of a 10kb sliding window with 1kb step sizes. Within each window, a chi-square goodness of fit test was applied to compare the proportion of SNPs that were significant at a test wise level of 0.01, to the proportion that would be expected by chance alone (0.05 for the methods used here). Resulting p-values that were less than 10^{-8} were truncated at that value.

[0256] Finally, the exons and introns of the genes in the covered region are plotted below each graph at the appropriate chromosomal positions. The gene boundary is indicated by the broken horizontal line. The exon positions are shown as thick, unbroken bars. An arrow is placed at the 3' end of each gene to show the direction of transcription.

Example 7

CASPR4 Region Proximal SNPs

[0257] It has been discovered that rs1395486 in the cell recognition protein CASPR4 gene is associated with occurrence of osteoarthritis in subjects. This gene product belongs to the neurexin family, members of which function in the nervous system as cell adhesion molecules and receptors. Like other neurexin proteins, CASPR4 contains epidermal growth factor repeats and laminin G domains. In addition, it includes an F5/8 type C domain, discoidin/neuropilin- and fibrinogen-like domains, and thrombospondin N-terminal-like domains. Alternative splicing of this gene results in 2 transcript variants encoding different isoforms. CASPR4 biological activity can be modulated by addition of an antibody, a recombinant binding partner, a binding agent, or a recombinant CASPR4 protein or functional fragment thereof.

[0258] Fifty-six additional allelic variants proximal to rs1395486 were identified and subsequently allelotyped in osteoarthritis case and control sample sets as described in Examples 1 and 2. The polymorphic variants are set forth in Table 28. The chromosome positions provided in column four of Table 28 are based on Genome “Build 34” of NCBI’s GenBank.

TABLE 28

dbSNP rs#	Chromosome	Position in SEQ ID NO: 6	Chromosome Position	Allele Variants
rs1896753	16	205	76177855	C/T
rs3974451	16	866	76178516	C/T
rs1820770	16	4212	76181862	C/T
rs1428753	16	5934	76183584	C/T
rs722229	16	11486	76189136	C/T
rs3851754	16	16969	76194619	A/G
rs2340430	16	22509	76200159	A/G
rs2340431	16	22796	76200446	A/G
rs1159415	16	28097	76205747	C/T
rs1506836	16	28626	76206276	C/T
rs1506837	16	28853	76206503	C/T
rs1506838	16	28873	76206523	C/T
rs966668	16	30155	76207805	A/G
rs1911245	16	30827	76208477	C/T
rs1506839	16	31956	76209606	C/T
rs1506840	16	32404	76210054	C/T
rs1876275	16	32944	76210594	A/G
rs1911242	16	35205	76212855	A/G
rs1911243	16	35227	76212877	C/T
rs981231	16	35781	76213431	C/T
rs1506829	16	41052	76218702	C/T

dbSNP rs#	Chromosome	Position in SEQ ID NO: 6	Chromosome Position	Allele Variants
rs1506833	16	45051	76222701	A/G
rs1395486	16	46039	76223689	C/T
rs1506832	16	47276	76224926	A/G
rs1506830	16	47678	76225328	C/T
rs968537	16	47716	76225366	A/G
rs1506816	16	51014	76228664	A/G
rs1506828	16	54408	76232058	A/G
rs1506827	16	54596	76232246	C/T
rs1542969	16	56853	76234503	C/G
rs1395484	16	61851	76239501	A/G
rs1876274	16	62016	76239666	A/G
rs1876273	16	62461	76240111	C/T
rs1506822	16	68257	76245907	C/G
rs1506820	16	69793	76247443	C/T
rs1506819	16	73976	76251626	A/C
rs1506818	16	73999	76251649	A/T
rs1506817	16	74053	76251703	A/G
rs1395488	16	75315	76252965	A/G
rs2221534	16	75729	76253379	G/T
rs1911244	16	76466	76254116	A/G
rs2135624	16	77216	76254866	C/T
rs2135623	16	77217	76254867	G/T
rs1506835	16	79239	76256889	C/G
rs1506834	16	80825	76258475	A/G
rs1995653	16	81060	76258710	C/G
rs1995652	16	81097	76258747	A/C
rs1395487	16	81426	76259076	G/T
rs3947083	16	84787	76262437	C/T
rs1506825	16	84896	76262546	A/T
rs1506824	16	85165	76262815	C/G
rs1567118	16	86502	76264152	C/G
rs1039683	16	86753	76264403	C/T
rs2879777	16	86941	76264591	C/T
rs1876272	16	88787	76266437	C/T
rs3035878	16	95598	76273248	-/AGAGC

Assay for Verifying and Allelotyping SNPs

[0259] The methods used to verify and allelotype the 56 proximal SNPs of Table 28 are the same methods described in Examples 1 and 2 herein. The primers and probes used in these assays are provided in Table 29 and Table 30, respectively.

TABLE 29

dbSNP rs#	Forward PCR primer	Reverse PCR primer
rs1896753	ACGTTGGATGTTTGAAGAGAGGGGACTAGAG	ACGTTGGATGGAAAATGAACTGGAATGGGG
rs3974451	ACGTTGGATGTTGCATAAGGTGTGAGGAAG	ACGTTGGATGAATGGTGTGGGAAAACCTGG
rs1820770	ACGTTGGATGCTTGAACCAACCCAAATGC	ACGTTGGATGGGCTGCATAGTATTCCACAG
rs1428753	ACGTTGGATGCAATAGCTATCTCCTACTTG	ACGTTGGATGGATGCTTTGTATTGACAACC
rs722229	ACGTTGGATGGAAGGAGGCTCACTATTTCC	ACGTTGGATGGGCTAGGGTAGCAAACATCA
rs3851754	ACGTTGGATGAGGTTTGGAGAATGCCAACT	ACGTTGGATGAGATTGAATCAGATGGACTG
rs2340430	ACGTTGGATGATGGCCTTCCAAAGATGTTT	ACGTTGGATGCATCTACAATCCCAATATGCC
rs2340431	ACGTTGGATGTTTGTGCAACCTCTGCAAGC	ACGTTGGATGAGATGTCAGCAGGATGCATG
rs1159415	ACGTTGGATGGCTTTCCAATGATTTGGGAG	ACGTTGGATGCTGGGTCTTCTTAATGTGTT
rs1506836	ACGTTGGATGCCTGGGCACAGATTCAATTC	ACGTTGGATGCTGCAGCGACCTTTCATTCA
rs1506837	ACGTTGGATGCTGACATTGAGCTAGTCTTTC	ACGTTGGATGGTAGTTGGTGAATTTGGTGG
rs1506838	ACGTTGGATGGTAGTTGGTGAATTTGGTGG	ACGTTGGATGGACATTGAGCTAGTCTTTC
rs966668	ACGTTGGATGCACCTCATAGTGTGAAAAGTC	ACGTTGGATGCCAGTAAATGCAAGATTTTCC
rs1911245	ACGTTGGATGAACAACCTAGGCAATTCGGTG	ACGTTGGATGCCATCAGAAGTAAACCGTTTC
rs1506839	ACGTTGGATGCCAAATTTTGTCTTGTAGAC	ACGTTGGATGTGCACAATTCAAGTGAAGTC
rs1506840	ACGTTGGATGGGAAGAATGACCTTGTGTGG	ACGTTGGATGAGCTGTGAGTGAGGATGATG
rs1876275	ACGTTGGATGAACTGTTCTCTGCCCTTTGG	ACGTTGGATGTTACGGACATAAGGGAAGG
rs1911242	ACGTTGGATGGTTCCCTAAGTACTTTAGAA	ACGTTGGATGCTCTGCAAAGCAATAAGCTAC
rs1911243	ACGTTGGATGCTTATAATTCAAGTCCCTAAG	ACGTTGGATGGCAATAAGCTACCAAAATAG
rs981231	ACGTTGGATGATGCTAACCTGTCTAAATCC	ACGTTGGATGTAGTGCTCTGGACTAGAAAAG
rs1506829	ACGTTGGATGTGGAAAGTTGCAATCCCTG	ACGTTGGATGCCATCTTAAAACCATGCGAG
rs1506833	ACGTTGGATGGTTTTATCTGGTTCCCTACAG	ACGTTGGATGGCTGTATACGTACTTTAAAC
rs1395486	ACGTTGGATGCTCATTTATTTATGTTTAC	ACGTTGGATGTGCTGGAATAATGATTGTTG
rs1506832	ACGTTGGATGGGTAATGGTCATAAGAATGCC	ACGTTGGATGGAGCTCAATTAGCATCTCTC
rs1506830	ACGTTGGATGCAACAGTAAAGGCATGAAAAG	ACGTTGGATGCATTGGACTATCAAAAAGTG
rs968537	ACGTTGGATGATTATTTGGTGGGAAGAGGG	ACGTTGGATGAAATGTTACGTAGGCCAAAC
rs1506816	ACGTTGGATGTACATATGACCACTGTTTCC	ACGTTGGATGCTAAGCAGGGAAGTAGTAAG
rs1506828	ACGTTGGATGGAGCTTTTTCCATTAGACCC	ACGTTGGATGGTTGAAAATCAGACAAGGGC
rs1506827	ACGTTGGATGAATGCGCTATATCTGATGAC	ACGTTGGATGAACCCATTTCTTAGCCAGAG
rs1542969	ACGTTGGATGCAGATTACAGCCAAGTTTGC	ACGTTGGATGGGTTTGAATTCCCAAGACAG
rs1395484	ACGTTGGATGCAAGCTCACATAACACAGGC	ACGTTGGATGAAGAGATGCCCCGATTTTGG
rs1876274	ACGTTGGATGGGTATCTGATCATCTGCCTG	ACGTTGGATGGGGATTGATTGACAAGGAG
rs1876273	ACGTTGGATGTGGAAGAAACATAGCTCCTG	ACGTTGGATGAAAATCCCTCCAGTGTTTGC
rs1506822	ACGTTGGATGTTCTCCAGATCTGCAAACAG	ACGTTGGATGGTAATGAGAGAAGTAGAGGC
rs1506820	ACGTTGGATGTTCTATATATGTGTGTGTGC	ACGTTGGATGTTAGGGTTCTCTAGAAAGAC
rs1506819	ACGTTGGATGTGAGGGAATTGTGTCTGCAG	ACGTTGGATGGCCAGAGAGGCTAGAAATTG
rs1506818	ACGTTGGATGAGGGCTGCTTAGCATTTTAC	ACGTTGGATGAGATCAGAGAGCAATGGTCC
rs1506817	ACGTTGGATGCCCTTTTCTCGTGCTTTCTC	ACGTTGGATGCTCAGATCCTTGGCCAATTC
rs1395488	ACGTTGGATGGACACTTGAATGCATCACCG	ACGTTGGATGGGTGACTTCTGTGACATTGC
rs2221534	ACGTTGGATGTAATGCAGGTCTCAAGTGCC	ACGTTGGATGCAAATCAGACTGAGTCGCTG
rs1911244	ACGTTGGATGACCTGTATTCTGTTCCAGG	ACGTTGGATGCAACATTCTACTTCTGGGGC
rs2135624	ACGTTGGATGGTACGCCCTACTCTCATATC	ACGTTGGATGAGCTCTTAATTCCATGGCAG
rs2135623	ACGTTGGATGGTACGCCCTACTCTCATATC	ACGTTGGATGAGCTCTTAATTCCATGGCAG
rs1506835	ACGTTGGATGAATTAGCTGGACATGGTGGC	ACGTTGGATGTCAAGTGAACCTCCAACCTC

dbSNP rs#	Forward PCR primer	Reverse PCR primer
rs1506834	ACGTTGGATGACATTTTCCCAGCACTGTCC	ACGTTGGATGCTCACTCCTACTCTGAGTAC
rs1995653	ACGTTGGATGCCAGCCTTCTGTTACTCTTG	ACGTTGGATGCTGTCCTCATGGTGTTC
rs1995652	ACGTTGGATGCGTGTTACAACCTGTAATGC	ACGTTGGATGACATAAATATGGCCCCTGTC
rs1395487	ACGTTGGATGAAAAGCTTTAGGTGCCACAG	ACGTTGGATGGCTTGTGTTACTTTAGCTAC
rs3947083	ACGTTGGATGAAGGTGGGCTCTTTATAGTG	ACGTTGGATGGAGGTGTGATGGTTATGTTTC
rs1506825	ACGTTGGATGCCTGCATATGATGTTCTGTG	ACGTTGGATGTAGCAGCTTTCGGTGTATAG
rs1506824	ACGTTGGATGAGCAATGGATTCAAATGCTC	ACGTTGGATGCACTGGTCGATGAAAAATAC
rs1567118	ACGTTGGATGTCGCCAATCTGTCCAAATG	ACGTTGGATGAATTGTCCCCGTTTCCACAG
rs1039683	ACGTTGGATGTGATGTGTGGAGGCATGTTG	ACGTTGGATGACAGGCAACAACCTGCCAAAG
rs2879777	ACGTTGGATGCTAATCATGTGCGATGAGGG	ACGTTGGATGAAGAAGAGATGGGCCATAGT
rs1876272	ACGTTGGATGTTCTTTGTCTGGAGTGGGAG	ACGTTGGATGGGTTCCAACACTAGCAGTTC
rs3035878	ACGTTGGATGTTCTACAAGGAGCTGTGTAG	ACGTTGGATGCTGACTGGTAAATTCACGAC

TABLE 30

dbSNP rs#	Extend Primer	Term Mix
rs1896753	GGAATTTAATTTGGTGCCTCTTCA	ACT
rs3974451	TTCAGTTTCAGCTTTCTGCATA	ACG
rs1820770	GAACCAACCCAAATGCCCATCA	ACT
rs1428753	TAACATTTACTGATAGAATAAAGC	ACT
rs722229	TTCCCTGCAGAAAATGAGACA	ACT
rs3851754	AACTCACACACACACACAGAA	ACT
rs2340430	CGTTGGGACCTATAGGTATG	ACT
rs2340431	CTCTGCAAGCTGGAAAGGAC	ACT
rs1159415	TATGTTTAGGAACATTTTCCTAAC	ACT
rs1506836	GTCTCACAGCTTGAAGATGC	ACG
rs1506837	CATTGAGCTAGTCTTTCCTCTGT	ACG
rs1506838	GTTGGTGAATTTGGTGGAGAATCT	ACT
rs966668	TCATAGTGTGAAAAGTCTAAAAAA	ACT
rs1911245	TTCCTCTTTTCAGACAAAATTG	ACG
rs1506839	AATTTTGCTTTGTTAGACCTTAGG	ACG
rs1506840	GCTGGTGCCTGTGAAATTG	ACG
rs1876275	TCTTGGTTCAGGTATCACCTA	ACG
rs1911242	TAGAAAAATTTGCCTTTTGAGAAA	ACG
rs1911243	TAATTCAGTTCCTAAGTACTTTA	ACT
rs981231	CCTGTCTAAATCCATTTGATTAAA	ACT
rs1506829	GATCTAAATAGCTACTGGGAAA	ACT
rs1506833	TCTGGTTCCTTACAGAAACACTTA	ACG
rs1395486	TTTCATGTTCAAAAAAATCTTCT	ACG
rs1506832	GGTCATAAGAATGCCATTATTCT	ACG
rs1506830	AATAATATGTTTGGCCTACGTAA	ACG
rs968537	AGGGAGGTAAGAGTCAACAGTAA	ACT

dbSNP rs#	Extend Primer	Term Mix
rs1506816	ATATGACCACTGTTTCCTCATT	ACT
rs1506828	CCATTAGACCCCTTAGCATAT	ACG
rs1506827	TGACAATAGAACTAAGACAAATA	ACT
rs1542969	GCCAAGTTTGCATCTTTCATGT	ACT
rs1395484	AACACAGGCACAGCTGTGAT	ACT
rs1876274	CTAATTCACAAATATTCCTTACT	ACT
rs1876273	TAGCTCCTGGCCCTACCAT	ACT
rs1506822	CTGCAAACAGGATCACTGCT	ACT
rs1506820	ATATACAGAACACACACACACA	ACG
rs1506819	TCTGCAGGAGCACGGACC	CGT
rs1506818	GGCCAAGGATCTGAGGGAA	CGT
rs1506817	TGCTTTCTCTAGGGCTGCTT	ACT
rs1395488	TGAATGCATCACCGGAGGAT	ACT
rs2221534	CTCAAGTGCCTATCTATCATG	CGT
rs1911244	TTCCAGGTTAGAATTCCAGAGAT	ACG
rs2135624	CTCTCATATCAATTCTCCCTGTT	ACG
rs2135623	CTCTCATATCAATTCTCCCTGT	ACT
rs1506835	GACATGGTGGCAAATTCCTGTA	ACT
rs1506834	ACTGTCCCATTCCTGTCATAA	ACT
rs1995653	TTCTGTACTCTTGATCAGAATGC	ACT
rs1995652	TAATGCTTTTATGAACTTAGTTGT	ACT
rs1395487	CTTTAGGTGCCACAGAAGATA	CGT
rs3947083	GGGCTCTTTATAGTGATTTTCCT	ACG
rs1506825	ATACTGTGAGAAAGATGAAGGT	CGT
rs1506824	CAAATGCTCAAATATCAATATGTG	ACT
rs1567118	TGTCCAAATGGCAATGTTGGT	ACT
rs1039683	GGAGGCATGTTGGAATTACAGAC	ACT
rs2879777	GAGGGGTGGTCACACAGC	ACT
rs1876272	CTGGAGTGGGAGACAGGGT	ACG
rs3035878	TGTGTAGCTAAATGTTGAGCAGAG	ACT

Genetic Analysis

[0260] Allelotyping results from the discovery cohort are shown for cases and controls in Table 31. The allele frequency for the A2 allele is noted in the fifth and sixth columns for osteoarthritis case pools and control pools, respectively, where “AF” is allele frequency. The allele frequency for the A1 allele can be easily calculated by subtracting the A2 allele frequency from 1 (A1 AF = 1-A2 AF). For example, the SNP rs1896753 has the following case and control allele frequencies: case A1 (A) = 0.79; case A2 (T) = 0.21; control A1 (A) = 0.81; and control A2 (T) = 0.19, where the nucleotide is provided in paranthesis. Some SNPs are labeled “untyped” because of failed assays.

TABLE 31

dbSNP rs#	Position in SEQ ID NO: 6	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs1896753	205	76177855	C/T			
rs3974451	866	76178516	C/T			
rs1820770	4212	76181862	C/T			
rs1428753	5934	76183584	C/T	0.486	0.467	0.459
rs722229	11486	76189136	C/T			
rs3851754	16969	76194619	A/G	0.287	0.300	0.569
rs2340430	22509	76200159	A/G	0.488	0.523	0.155
rs2340431	22796	76200446	A/G	0.030	0.028	0.844
rs1159415	28097	76205747	C/T	0.480	0.477	0.919
rs1506836	28626	76206276	C/T	0.401	0.404	0.891
rs1506837	28853	76206503	C/T	0.394	0.396	0.933
rs1506838	28873	76206523	C/T	0.334	0.343	0.727
rs966668	30155	76207805	A/G			
rs1911245	30827	76208477	C/T	0.836	0.824	0.631
rs1506839	31956	76209606	C/T	0.434	0.436	0.936
rs1506840	32404	76210054	C/T	0.382	0.381	0.993
rs1876275	32944	76210594	A/G	0.463	0.461	0.918
rs1911242	35205	76212855	A/G	0.419	0.410	0.703
rs1911243	35227	76212877	C/T			
rs981231	35781	76213431	C/T	0.451	0.430	0.510
rs1506829	41052	76218702	C/T	0.393	0.379	0.576
rs1506833	45051	76222701	A/G	0.509	0.530	0.378
rs1395486	46039	76223689	C/T			
rs1506832	47276	76224926	A/G	0.518	0.516	0.949
rs1506830	47678	76225328	C/T	0.036	0.031	0.710
rs968537	47716	76225366	A/G	0.243	0.275	0.175
rs1506816	51014	76228664	A/G	0.392	0.369	0.348
rs1506828	54408	76232058	A/G	0.418	0.413	0.816
rs1506827	54596	76232246	C/T	0.432	0.449	0.477
rs1542969	56853	76234503	C/G			
rs1395484	61851	76239501	A/G	0.417	0.441	0.349
rs1876274	62016	76239666	A/G	0.381	0.369	0.629
rs1876273	62461	76240111	C/T	0.382	0.364	0.445
rs1506822	68257	76245907	C/G	0.355	0.351	0.855
rs1506820	69793	76247443	C/T	0.326	0.256	0.054
rs1506819	73976	76251626	A/C	0.446	0.424	0.358
rs1506818	73999	76251649	A/T	0.126	0.145	0.465
rs1506817	74053	76251703	A/G	0.186	0.199	0.570
rs1395488	75315	76252965	A/G	0.489	0.499	0.689
rs2221534	75729	76253379	G/T	0.450	0.431	0.455
rs1911244	76466	76254116	A/G	0.493	0.491	0.960
rs2135624	77216	76254866	C/T			
rs2135623	77217	76254867	G/T	0.034	0.032	0.899
rs1506835	79239	76256889	C/G	0.549	0.538	0.666
rs1506834	80825	76258475	A/G	0.390	0.392	0.958
rs1995653	81060	76258710	C/G	0.396	0.402	0.783
rs1995652	81097	76258747	A/C	0.436	0.435	0.979
rs1395487	81426	76259076	G/T	0.505	0.504	0.975
rs3947083	84787	76262437	C/T	0.373	0.366	0.773
rs1506825	84896	76262546	A/T	0.412	0.398	0.569
rs1506824	85165	76262815	C/G	0.444	0.414	0.242
rs1567118	86502	76264152	C/G	0.032	0.024	0.557
rs1039683	86753	76264403	C/T	0.382	0.373	0.707
rs2879777	86941	76264591	C/T	0.269	0.279	0.676

dbSNP rs#	Position in SEQ ID NO: 6	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs1876272	88787	76266437	C/T			
rs3035878	95598	76273248	-/AGAGC	0.978	untyped	NA

[0261] The *CASPR4* proximal SNPs were also allelotyped in the replication cohorts using the methods described herein and the primers provided in Tables 29 and 30. The replication allelotyping results for replication cohort #1 and replication cohort #2 are provided in Tables 32 and 33, respectively.

TABLE 32

dbSNP rs#	Position in SEQ ID NO: 6	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs1896753	205	76177855	C/T			
rs3974451	866	76178516	C/T			
rs1820770	4212	76181862	C/T			
rs1428753	5934	76183584	C/T	0.463	0.474	0.756
rs722229	11486	76189136	C/T			
rs3851754	16969	76194619	A/G	0.283	0.309	0.375
rs2340430	22509	76200159	A/G	0.494	0.519	0.477
rs2340431	22796	76200446	A/G	0.035	0.028	0.748
rs1159415	28097	76205747	C/T	0.436	0.472	0.287
rs1506836	28626	76206276	C/T	0.392	0.401	0.786
rs1506837	28853	76206503	C/T	0.388	0.399	0.727
rs1506838	28873	76206523	C/T	0.318	0.327	0.778
rs966668	30155	76207805	A/G			
rs1911245	30827	76208477	C/T	0.825	0.821	0.896
rs1506839	31956	76209606	C/T	0.450	0.441	0.817
rs1506840	32404	76210054	C/T	0.379	0.383	0.926
rs1876275	32944	76210594	A/G	0.469	0.470	0.986
rs1911242	35205	76212855	A/G	0.437	0.415	0.514
rs1911243	35227	76212877	C/T			
rs981231	35781	76213431	C/T	0.449	0.414	0.415
rs1506829	41052	76218702	C/T	0.398	0.394	0.894
rs1506833	45051	76222701	A/G	0.515	0.544	0.393
rs1395486	46039	76223689	C/T			
rs1506832	47276	76224926	A/G	0.526	0.511	0.720
rs1506830	47678	76225328	C/T	0.053	0.039	0.488
rs968537	47716	76225366	A/G	0.241	0.298	0.045
rs1506816	51014	76228664	A/G	0.379	0.370	0.771
rs1506828	54408	76232058	A/G	0.416	0.429	0.706
rs1506827	54596	76232246	C/T	0.428	0.435	0.836
rs1542969	56853	76234503	C/G			
rs1395484	61851	76239501	A/G	0.418	0.459	0.208
rs1876274	62016	76239666	A/G	0.384	0.382	0.942
rs1876273	62461	76240111	C/T	0.393	0.360	0.271
rs1506822	68257	76245907	C/G	0.353	0.368	0.637
rs1506820	69793	76247443	C/T	0.288	untyped	NA
rs1506819	73976	76251626	A/C	0.453	0.424	0.378
rs1506818	73999	76251649	A/T	0.149	NA	0.126
rs1506817	74053	76251703	A/G	0.195	0.212	0.573
rs1395488	75315	76252965	A/G	0.490	0.490	1.000

dbSNP rs#	Position in SEQ ID NO: 6	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs2221534	75729	76253379	G/T	0.446	0.433	0.711
rs1911244	76466	76254116	A/G	0.495	0.480	0.646
rs2135624	77216	76254866	C/T			
rs2135623	77217	76254867	G/T	0.027	0.030	0.896
rs1506835	79239	76256889	C/G	0.563	0.556	0.848
rs1506834	80825	76258475	A/G	0.377	0.388	0.722
rs1995653	81060	76258710	C/G	0.381	0.395	0.675
rs1995652	81097	76258747	A/C	0.435	0.423	0.750
rs1395487	81426	76259076	G/T	0.505	0.500	0.874
rs3947083	84787	76262437	C/T	0.367	0.370	0.929
rs1506825	84896	76262546	A/T	0.406	0.397	0.798
rs1506824	85165	76262815	C/G	0.446	0.413	0.361
rs1567118	86502	76264152	C/G	0.029	0.023	0.776
rs1039683	86753	76264403	C/T	0.376	0.365	0.729
rs2879777	86941	76264591	C/T	0.265	0.278	0.669
rs1876272	88787	76266437	C/T			
rs3035878	95598	76273248	-/AGAGC	0.972	untyped	NA

TABLE 33

dbSNP rs#	Position in SEQ ID NO: 6	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs1896753	205	76177855	C/T			
rs3974451	866	76178516	C/T			
rs1820770	4212	76181862	C/T			
rs1428753	5934	76183584	C/T	0.515	0.457	0.124
rs722229	11486	76189136	C/T			
rs3851754	16969	76194619	A/G	0.292	0.286	0.868
rs2340430	22509	76200159	A/G	0.480	0.531	0.169
rs2340431	22796	76200446	A/G	0.024	0.027	0.900
rs1159415	28097	76205747	C/T	0.535	0.485	0.252
rs1506836	28626	76206276	C/T	0.412	0.410	0.947
rs1506837	28853	76206503	C/T	0.402	0.391	0.768
rs1506838	28873	76206523	C/T	0.355	0.368	0.734
rs966668	30155	76207805	A/G			
rs1911245	30827	76208477	C/T	0.849	0.828	0.569
rs1506839	31956	76209606	C/T	0.414	0.428	0.746
rs1506840	32404	76210054	C/T	0.384	0.379	0.905
rs1876275	32944	76210594	A/G	0.456	0.447	0.805
rs1911242	35205	76212855	A/G	0.397	0.402	0.892
rs1911243	35227	76212877	C/T			
rs981231	35781	76213431	C/T	0.454	0.455	0.971
rs1506829	41052	76218702	C/T	0.386	0.356	0.424
rs1506833	45051	76222701	A/G	0.500	0.509	0.811
rs1395486	46039	76223689	C/T			
rs1506832	47276	76224926	A/G	0.508	0.524	0.689
rs1506830	47678	76225328	C/T			
rs968537	47716	76225366	A/G	0.246	0.237	0.806
rs1506816	51014	76228664	A/G	0.408	0.367	0.284
rs1506828	54408	76232058	A/G	0.421	0.387	0.358
rs1506827	54596	76232246	C/T	0.436	0.471	0.346
rs1542969	56853	76234503	C/G			
rs1395484	61851	76239501	A/G	0.416	0.413	0.938

dbSNP rs#	Position in SEQ ID NO: 6	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs1876274	62016	76239666	A/G	0.376	0.350	0.447
rs1876273	62461	76240111	C/T	0.367	0.370	0.924
rs1506822	68257	76245907	C/G	0.358	0.325	0.355
rs1506820	69793	76247443	C/T	0.373	0.256	0.007
rs1506819	73976	76251626	A/C	0.438	0.424	0.703
rs1506818	73999	76251649	A/T	0.139	-0.013	
rs1506817	74053	76251703	A/G	0.174	0.178	0.897
rs1395488	75315	76252965	A/G	0.487	0.512	0.505
rs2221534	75729	76253379	G/T	0.455	0.429	0.463
rs1911244	76466	76254116	A/G	0.489	0.509	0.581
rs2135624	77216	76254866	C/T			
rs2135623	77217	76254867	G/T	0.042	0.035	0.748
rs1506835	79239	76256889	C/G	0.531	0.510	0.562
rs1506834	80825	76258475	A/G	0.407	0.397	0.787
rs1995653	81060	76258710	C/G	0.414	0.413	0.984
rs1995652	81097	76258747	A/C	0.437	0.455	0.629
rs1395487	81426	76259076	G/T	0.506	0.512	0.869
rs3947083	84787	76262437	C/T	0.379	0.359	0.559
rs1506825	84896	76262546	A/T	0.419	0.399	0.579
rs1506824	85165	76262815	C/G	0.442	0.414	0.471
rs1567118	86502	76264152	C/G	0.036	0.025	0.574
rs1039683	86753	76264403	C/T	0.389	0.385	0.910
rs2879777	86941	76264591	C/T	0.275	0.280	0.883
rs1876272	88787	76266437	C/T			
rs3035878	95598	76273248	-/AGAGC	untyped	0.980	NA

[0262] Allelotyping results were considered particularly significant with a calculated p-value of less than or equal to 0.05 for allelotype results. These values are indicated in bold. The allelotyping p-values were plotted in Figure 1F for the discovery cohort. The position of each SNP on the chromosome is presented on the x-axis. The y-axis gives the negative logarithm (base 10) of the p-value comparing the estimated allele in the case group to that of the control group. The minor allele frequency of the control group for each SNP designated by an X or other symbol on the graphs in Figure 1F can be determined by consulting Table 31. For example, the left-most X on the left graph is at position 76177855. By proceeding down the Table from top to bottom and across the graphs from left to right the allele frequency associated with each symbol shown can be determined.

[0263] To aid the interpretation, multiple lines have been added to the graph. The broken horizontal lines are drawn at two common significance levels, 0.05 and 0.01. The vertical broken lines are drawn every 20kb to assist in the interpretation of distances between SNPs. Two other lines are drawn to expose linear trends in the association of SNPs to the disease. The light gray line (or generally bottom-most curve) is a nonlinear smoother through the data points on the graph using a local polynomial regression method (W.S. Cleveland, E. Grosse and W.M. Shyu (1992) Local regression models. Chapter 8 of Statistical Models in S eds J.M. Chambers and T.J. Hastie, Wadsworth & Brooks/Cole.). The black

line provides a local test for excess statistical significance to identify regions of association. This was created by use of a 10kb sliding window with 1kb step sizes. Within each window, a chi-square goodness of fit test was applied to compare the proportion of SNPs that were significant at a test wise level of 0.01, to the proportion that would be expected by chance alone (0.05 for the methods used here). Resulting p-values that were less than 10^{-8} were truncated at that value.

[0264] Finally, the exons and introns of the genes in the covered region are plotted below each graph at the appropriate chromosomal positions. The gene boundary is indicated by the broken horizontal line. The exon positions are shown as thick, unbroken bars. An arrow is placed at the 3' end of each gene to show the direction of transcription.

Example 8

APOB Proximal SNPs

[0265] It has been discovered that rs1367117 is associated with occurrence of osteoarthritis in subjects. The polymorphic variant lies within the *APOB* gene and codes for a I98T amino acid change. The guanine allele of SNP rs1367117 is associated with osteoarthritis (see Table 5) and codes for a threonine at position 98 (see e.g. amino acid sequence in SEQ ID NO: 19).

[0266] Apolipoprotein B (ApoB) is the main apolipoprotein of chylomicrons and low density lipoproteins (LDL). Apo B binds to sulfated proteoglycans, especially chondroitin and dermatan sulfate, that are components of cartilage (Camejo et. al., *Atherosclerosis*. 1998 Aug;139(2):205-22). This may contribute to inflammation/joint damage by lipoprotein oxidation products. In addition, increased levels of ApoB is seen as a risk factor for osteonecrosis (Miyashita et. al., *Ann Rheum Dis*. 1999 Aug;58(8):514-6). Lipoprotein deposition has been noted in inflammatory (rheumatoid) arthritis and may play a role in inflammation mediated osteoarthritis. ApoB function can be modulated by addition of an antibody or a decoy receptor for ApoB. Examples of antibodies and small molecules that specifically interact with ApoB are described in U.S. Patent Nos. 6,107,045; 6,309,844; 5,330,910; and 6,369,075.

[0267] One hundred twenty-two additional allelic variants proximal to rs1367117 were identified and subsequently allelotyped in osteoarthritis case and control sample sets as described in Examples 1 and 2. The polymorphic variants are set forth in Table 34. The chromosome positions provided in column four of Table 34 are based on Genome "Build 34" of NCBI's GenBank.

TABLE 34

dbSNP rs#	Chromosome	Position in SEQ ID NO: 1	Chromosome Position	Allele Variants
rs1318006	2	238	21188688	C/T
rs1318005	2	294	21188744	C/T

dbSNP rs#	Chromosome	Position in SEQ ID NO: 1	Chromosome Position	Allele Variants
rs1318004	2	295	21188745	A/G
rs1318003	2	347	21188797	A/C
rs4327259	2	1425	21189875	A/C
rs6756501	2	4891	21193341	C/T
rs6725189	2	5087	21193537	G/T
rs4665709	2	7041	21195491	A/G
rs4665710	2	7121	21195571	A/C
rs4371387	2	7219	21195669	A/G
rs952274	2	7443	21195893	G/T
rs952275	2	7485	21195935	G/T
rs1801695	2	10939	21199389	A/G
rs1042034	2	11367	21199817	A/G
rs1801702	2	11571	21200021	C/G
rs1042031	2	11839	21200289	A/G
rs2678378	2	12551	21201001	A/G
rs2678379	2	12646	21201096	A/G
rs1800479	2	13469	21201919	G/C
rs1801701	2	14913	21203363	A/G
rs4362589	2	15205	21203655	G/T
rs5742904	2	15246	21203696	A/G
rs1799812	2	15695	21204145	G/A
rs2163204	2	17473	21205923	G/T
rs676210	2	17610	21206060	A/G
rs1042006	2	17828	21206278	A/C
rs1801696	2	18130	21206580	A/G
rs693	2	18281	21206731	C/T
rs1041974	2	18623	21207073	C/G
rs1041968	2	18890	21207340	C/T
rs568413	2	21561	21210011	C/T
rs2854726	2	23100	21211550	A/T
rs2854725	2	23872	21212322	A/C
rs2000998	2	24581	21213031	A/T
rs2000997	2	24582	21213032	A/T
rs497166	2	24983	21213433	C/T
rs562956	2	27540	21215990	A/T
rs7589300	2	30846	21219296	C/T
rs3791980	2	31415	21219865	G/T
rs3791981	2	31453	21219903	A/G
rs1801700	2	31899	21220349	T/C
rs679899	2	37000	21225450	A/G
rs1041952	2	38681	21227131	C/G
rs6727706	2	39287	21227737	C/T
rs6719207	2	42951	21231401	A/T
rs1469513	2	45648	21234098	C/T
rs1800478	2	46222	21234672	C/T
rs550619	2	46687	21235137	A/G

dbSNP rs#	Chromosome	Position in SEQ ID NO: 1	Chromosome Position	Allele Variants
rs6752026	2	47020	21235470	A/G
rs579826	2	47593	21236043	C/T
rs597331	2	48513	21236963	C/T
rs1367116	2	49723	21238173	A/G
rs1367117	2	49986	21238436	A/G
rs1800480	2	53018	21241468	C/G
rs1800481	2	53296	21241746	C/T
rs934197	2	53547	21241997	A/G
rs1625764	2	53899	21242349	C/T
rs1625714	2	53916	21242366	G/T
rs1560357	2	53933	21242383	A/C
rs617314	2	54305	21242755	G/T
rs547186	2	55327	21243777	A/T
rs589566	2	55895	21244345	C/T
rs588245	2	56143	21244593	C/T
rs585967	2	56640	21245090	G/T
rs7562777	2	58486	21246936	A/G
rs7575840	2	59576	21248026	G/T
rs7567653	2	63048	21251498	A/G
rs6548010	2	64008	21252458	A/G
rs6548011	2	64018	21252468	C/T
rs934198	2	64859	21253309	A/C
rs634292	2	65995	21254445	G/T
rs1003177	2	66905	21255355	A/G
rs6726115	2	67183	21255633	A/G
rs481069	2	67942	21256392	C/T
rs1367115	2	68101	21256551	A/G
rs666126	2	68521	21256971	A/G
rs7566030	2	68664	21257114	C/G
rs7590135	2	68988	21257438	A/G
rs6718513	2	69178	21257628	C/G
rs515135	2	72143	21260593	A/G
rs1367114	2	74183	21262633	C/G
rs563290	2	74312	21262762	C/T
rs562338	2	74407	21262857	C/T
rs581411	2	75518	21263968	A/G
rs580889	2	76153	21264603	A/G
rs548145	2	77398	21265848	A/G
rs668948	2	77615	21266065	A/G
rs594677	2	79092	21267542	C/T
rs571468	2	80000	21268450	G/T
rs4665492	2	80125	21268575	A/C
rs622236	2	80595	21269045	G/T
rs541041	2	81061	21269511	C/T
rs540156	2	81151	21269601	A/G
rs1367113	2	81918	21270368	C/T

dbSNP rs#	Chromosome	Position in SEQ ID NO: 1	Chromosome Position	Allele Variants
rs1897084	2	83072	21271522	C/T
rs1897083	2	83137	21271587	C/T
rs478588	2	83235	21271685	C/T
rs664894	2	83263	21271713	A/T
rs1594286	2	83279	21271729	A/G
rs7422168	2	83280	21271730	C/G
rs565202	2	83533	21271983	C/T
rs1429974	2	86856	21275306	G/T
rs5829769	2	87186	21275636	-/TATA
rs3056575	2	87189	21275639	-/ATAT
rs6708168	2	87727	21276177	A/T
rs6756743	2	87978	21276428	C/T
rs2195598	2	89129	21277579	A/G
rs7567217	2	89556	21278006	C/T
rs568938	2	89702	21278152	A/G
rs666416	2	90233	21278683	A/G
rs6761300	2	93060	21281510	A/G
rs5829770	2	94779	21283229	-/T
rs1429973	2	95367	21283817	A/G
rs1429972	2	95844	21284294	A/G
rs6756284	2	95942	21284392	A/G
rs749988	2	96884	21285334	C/T
rs749987	2	96938	21285388	A/G
rs754524	2	97627	21286077	A/C
rs754523	2	97777	21286227	C/T
rs675430	2	97871	21286321	A/C
rs600012	2	98746	21287196	A/G
rs614303	2	99663	21288113	A/G

Assay for Verifying and Allelotyping SNPs

[0268] The methods used to verify and allelotype the 122 proximal SNPs of Table 34 are the same methods described in Examples 1 and 2 herein. The primers and probes used in these assays are provided in Table 35 and Table 36, respectively.

TABLE 35

dbSNP rs#	Forward PCR primer	Reverse PCR primer
rs1318006	ACGTTGGATGTCTCATGGCCCATCCAAGGC	ACGTTGGATGAAGGAGCCCATGAAGGCAGC
rs1318005	ACGTTGGATGACAGCCTTGGATGGGCCATG	ACGTTGGATGTCTCCAGTCTGGTGGAAAG
rs1318004	ACGTTGGATGACAGCCTTGGATGGGCCATG	ACGTTGGATGTCTCCAGTCTGGTGGAAAG
rs1318003	ACGTTGGATGTTTCCACCAGACTGGGAGAC	ACGTTGGATGAGTGCCAGCACAGAGTCTT
rs4327259	ACGTTGGATGAACAAGCTTGCTCAGCCACT	ACGTTGGATGTGTGTTCTGTCCAGGAAGAG
rs6756501	ACGTTGGATGATGCATTCATTCGCTGTTTG	ACGTTGGATGGAGATCAATGAGAAAAATAGG

dbSNP rs#	Forward PCR primer	Reverse PCR primer
rs6725189	ACGTTGGATGAAGAACAATAGAGAGGGCCG	ACGTTGGATGAGTATTGACTGCCTTGGTTC
rs4665709	ACGTTGGATGGCACAACCTCATAGATGTGG	ACGTTGGATGCCACCTCCATCATTGTGGAT
rs4665710	ACGTTGGATGAATCCACAATGATGGAGGTG	ACGTTGGATGGATAACTCACTCACTATCACG
rs4371387	ACGTTGGATGTAAAAGTGTGTAGCACCTCC	ACGTTGGATGTCATGGCAGAAGTTAAAGGG
rs952274	ACGTTGGATGCAGAAGGGTGACATGCATTG	ACGTTGGATGCTCATATCCAGATTCACCCC
rs952275	ACGTTGGATGCACAATGCATGTCACCTTC	ACGTTGGATGGACACTCTCTTTGCTGAAGG
rs1801695	ACGTTGGATGGAAATTATTTTCTTCGTCG	ACGTTGGATGTGCTCAGGAAATAATTA
rs1042034	ACGTTGGATGATCCAAGATGAGATCAACAC	ACGTTGGATGGGCATAGGTTTTCTTTCAAC
rs1801702	ACGTTGGATGTTTTGATAAATCTTTCAAC	ACGTTGGATGCTAATAGATGTAATCTCGA
rs1042031	ACGTTGGATGGTTTGATGGCTTGGTACGAG	ACGTTGGATGTTTCCCCGGAAACTGGAATC
rs2678378	ACGTTGGATGGTTTTAGTCCTAGGAAGGC	ACGTTGGATGTATCACATGCCCCAGAAAGG
rs2678379	ACGTTGGATGCTTCCTAGGACTGAAACTG	ACGTTGGATGTGGGCTCCAACCTTGCTTTT
rs1800479	ACGTTGGATGAAGGGTATGGAGATGAAGA	ACGTTGGATGACCTTATACCTTTTGA
rs1801701	ACGTTGGATGCTTGGTCATTGGAAAGCTCG	ACGTTGGATGGTGGCCCTGAATGCTAACAC
rs4362589	ACGTTGGATGCTGCAGGGCACTTCCAAAAT	ACGTTGGATGTATATGCGTTGGAGTGTGGC
rs5742904	ACGTTGGATGATTTTGAAGTGCCCTGCAG	ACGTTGGATGCTATTGCTAGTGAGGCCAAC
rs1799812	ACGTTGGATGTTGTGGTGCCCTCTAATTT	ACGTTGGATGCATCTTCATCTGTCAATTGA
rs2163204	ACGTTGGATGTTTGGACTCTCCTTTGGCAG	ACGTTGGATGGCTGACATAGGGAATGGAAC
rs676210	ACGTTGGATGCCCAACTCTCAACCTTAATG	ACGTTGGATGAATTGTGTGTGAGATGTGGG
rs1042006	ACGTTGGATGCAGCATCTGGTCAATGGTTC	ACGTTGGATGACACCTTCCACATTCCTTCC
rs1801696	ACGTTGGATGTGCTAAGAACCCTACTGAC	ACGTTGGATGGCCCAATCTTGGATAGAAT
rs693	ACGTTGGATGCAGCATCTTTGGCTCACATG	ACGTTGGATGTCCTGCTGAATGTCCATTTG
rs1041974	ACGTTGGATGTACTTTGAGAAATTGGTTGG	ACGTTGGATGGTTAACATCTTCAATGAATG
rs1041968	ACGTTGGATGTCAGCTACTTCAAATCCCC	ACGTTGGATGGGCTATTGATGTTAGAGTGC
rs568413	ACGTTGGATGGAGACTGGGTTGTTTCCAAG	ACGTTGGATGCCACAAGAATACGTTACACAC
rs2854726	ACGTTGGATGCTCTAGCTTAACAGCAAGCC	ACGTTGGATGGCAAATTCTCCCTCTGACTG
rs2854725	ACGTTGGATGCATTCAGCTTTGTGTAAGT	ACGTTGGATGTTTCAAAGACTGTATAAGG
rs2000998	ACGTTGGATGTGAACCATCCTTGATCTGG	ACGTTGGATGTGGCACCAGATGATTTGTCC
rs2000997	ACGTTGGATGTGGCACCAGATGATTTGTCC	ACGTTGGATGTGAACCATCCTTGATCTGG
rs497166	ACGTTGGATGTCCCAAAGTGCTGGGATTAC	ACGTTGGATGAAATCCAAGTGGACATGCGC
rs562956	ACGTTGGATGTAACAGTCTTACCACACGGC	ACGTTGGATGATAAGGGAAAGTCTCCCTGG
rs7589300	ACGTTGGATGTACCACGTATGTTGAGTGAG	ACGTTGGATGCCCTTACTCTATGATTACTGC
rs3791980	ACGTTGGATGTCTGGAGAGATCATCTTTGG	ACGTTGGATGCTACCTAGCTACCTCAAATC
rs3791981	ACGTTGGATGTGTTTTGAGAATGAAGAAAC	ACGTTGGATGGTTCTTAGGTATTTTTGGG
rs1801700	ACGTTGGATGGACCCGACTCGTGGAAGAA	ACGTTGGATGTCGCTAGGAGTGGGGTCCA
rs679899	ACGTTGGATGCTGAAGTCCATGACAGTTGG	ACGTTGGATGTTGTGGCTTCCCATATTGCC
rs1041952	ACGTTGGATGCATGGAGCAGTTAACTCCAG	ACGTTGGATGTCTGGATCATCAGTGATGGC
rs6727706	ACGTTGGATGGCACCCTTATTGAAAAGGG	ACGTTGGATGCACATACTTACAGTCAACGG
rs6719207	ACGTTGGATGGTCCCAGTTGTAACCATGTC	ACGTTGGATGGGAATCCAGACTTGTCTGAG
rs1469513	ACGTTGGATGCTTTTCTGCACAAGGACTCC	ACGTTGGATGACTCCACTTCATGGGATGAG
rs1800478	ACGTTGGATGTGACGGTAAAGTGAGTGGAG	ACGTTGGATGCCCGTGTGAATACATGTGG
rs550619	ACGTTGGATGGCAAACACAGGTGAAGCATC	ACGTTGGATGGGCTTATCAGGTTGGGTCTA
rs6752026	ACGTTGGATGCAGAAGGGAAGCAGGTTTT	ACGTTGGATGCAGAAATGATGCCCTCTTG
rs579826	ACGTTGGATGAAAGTGCTGGGACTACAGGC	ACGTTGGATGATATGGGTGGAGAACAGAGC
rs597331	ACGTTGGATGACACTCTCTCAGAAAGTTCC	ACGTTGGATGGTATGGTGATCAGATCAGAG
rs1367116	ACGTTGGATGCAAGAAGTTAAAGCATGAG	ACGTTGGATGATCATCAAAAAGAGAGAAGC

dbSNP rs#	Forward PCR primer	Reverse PCR primer
rs1367117	ACGTTGGATGTTGGTTTTCTTCAGCAAGGC	ACGTTGGATGAGCTTCATCCTGAAGACCAG
rs1800480	ACGTTGGATGCCGAGAAGGGCACTCAGCC	ACGTTGGATGCGCCGGCCGCGCATTCCCA
rs1800481	ACGTTGGATGATCTGAAGAAGGCACCCCTG	ACGTTGGATGAAGCGTCTTCAGTGCTCTGG
rs934197	ACGTTGGATGTGACTGGTCACTCACCAGAC	ACGTTGGATGATCCTGATCAGAATCTGTGG
rs1625764	ACGTTGGATGCAGAGGCATCGAGCGCTGG	ACGTTGGATGGACAGGACACGTCATGTTCC
rs1625714	ACGTTGGATGAATTCCACTACCGCTGATTC	ACGTTGGATGATCGTTTCCTTCTCTCTAG
rs1560357	ACGTTGGATGGTCCCTGAAATTCCACTACC	ACGTTGGATGATTTCCACCGGAAGCTTCA
rs617314	ACGTTGGATGCAGTCTTCACCAGTAGCTTG	ACGTTGGATGTTGCAGAAAGTCAGTGTGTGC
rs547186	ACGTTGGATGCAGTTCAGGGAAGACTTGCC	ACGTTGGATGGAGAGGACTGTCACCATCTC
rs589566	ACGTTGGATGCCAGCAGACCAATATTCTG	ACGTTGGATGGGTATAGCTGAATGGTGCAG
rs588245	ACGTTGGATGGCTCCAAAATCTCATCTGGC	ACGTTGGATGAGCTTCTGGGCATCATTTGC
rs585967	ACGTTGGATGTGACAGGGAATCAGAGTCAC	ACGTTGGATGCCACCTACTGCACTGAATCT
rs7562777	ACGTTGGATGTTGGAGATTGCTCTTTGGGC	ACGTTGGATGTGACCTCAGGTTATCCACAC
rs7575840	ACGTTGGATGCATAGACTGTCCATCACAGG	ACGTTGGATGGGTGTCAGAAAACTTCCAC
rs7567653	ACGTTGGATGAAAGTGGTGATGGATGCCTG	ACGTTGGATGGGGAGCAAATAGCTCATCTG
rs6548010	ACGTTGGATGGCCTGGATTGCGGTTTTTAA	ACGTTGGATGCTATAAGCTGCTTATCAGAG
rs6548011	ACGTTGGATGCTATAAGCTGCTTATCAGAG	ACGTTGGATGGCCTGGATTGCGGTTTTTAA
rs934198	ACGTTGGATGACATGGAAGGAGGATGAGTG	ACGTTGGATGAGGTAGGACCCTCATGATTG
rs634292	ACGTTGGATGGAGGCTTGTTTATGGCACAG	ACGTTGGATGCGTGCTTTTTCTCAAGTGCC
rs1003177	ACGTTGGATGTACACAGACCCAGAAGATAC	ACGTTGGATGGATGCATGAACAAAGGAAGC
rs6726115	ACGTTGGATGATACAGATAAGGCACTTGCC	ACGTTGGATGAGGGAAGTGAACGTGAAAGG
rs481069	ACGTTGGATGTTTGAACCTTCCTGAATGGTG	ACGTTGGATGATTGTGAGGGTTTACTTTCC
rs1367115	ACGTTGGATGGGTTTGGAACAAGTATTGG	ACGTTGGATGGGTAGGGAAGTACTTTCAACG
rs666126	ACGTTGGATGTTCTGCAGGATTCATCTCTC	ACGTTGGATGTTTTGTATGCCAGGTTAAGG
rs7566030	ACGTTGGATGGATACAGAAGAGAGTGGTGG	ACGTTGGATGAGACTTGAGCCTTCAATGGC
rs7590135	ACGTTGGATGACTGGTCTTAGGGTTACACC	ACGTTGGATGACAAAGCACCTGCTCCAAGA
rs6718513	ACGTTGGATGCTTCCCTAGGTCTGAAGAAC	ACGTTGGATGGCTTCTTTAGTGCCAAAGAG
rs515135	ACGTTGGATGGGCTTACAGCCAAGTAACAG	ACGTTGGATGACCATCTTGTTACTGCACAG
rs1367114	ACGTTGGATGGTTGGAGAATTATTTGCAGG	ACGTTGGATGGTGTGTGTGTATTTGTGTTG
rs563290	ACGTTGGATGGGGAAAATGCTGCAATGAAC	ACGTTGGATGTCTGGGTATTCATCCAGAAG
rs562338	ACGTTGGATGACCCAAGATGTAGAAACAGC	ACGTTGGATGCCATGGTTTGCATACATCAC
rs581411	ACGTTGGATGACCTGGTGTGCTTAACTGTT	ACGTTGGATGGACAAGTGAAAAAGTTGGGC
rs580889	ACGTTGGATGTGGGCTGACTCTTATCTC	ACGTTGGATGCCTCTGAAGTGAATAAGCC
rs548145	ACGTTGGATGGAAGGAGGATGGTCAGAAAC	ACGTTGGATGAGCTGTATCTCCCCTTTGTG
rs668948	ACGTTGGATGATTGGAATAGGAAGGGCATG	ACGTTGGATGCTCTATCGTAATGGGGAAAAG
rs594677	ACGTTGGATGGACTTGGTATTGAACAGGAC	ACGTTGGATGTAGCAGGCATTTGCACTTTG
rs571468	ACGTTGGATGGTGATGAAATTAAGGCCAGG	ACGTTGGATGATCTCACTGTTTCTCCAGGG
rs4665492	ACGTTGGATGAGTGCGTCACCTTCTATTGAC	ACGTTGGATGCCAACAAGCATGTAAGTCAC
rs622236	ACGTTGGATGCGCTTTTCTGTACTGTTTGAG	ACGTTGGATGTCCCTTGCTACTACAAAGAC
rs541041	ACGTTGGATGGAGAGGAAAAGGTCACATTC	ACGTTGGATGATGCAGTAAGAGTAAGTGGC
rs540156	ACGTTGGATGCTTGTCTTTGAAATTCATAG	ACGTTGGATGCTCTCCTCCATGAATAATTAC
rs1367113	ACGTTGGATGATAATACTGCAGGAGGACAG	ACGTTGGATGAGAACAAATGTCCTTCTCTG
rs1897084	ACGTTGGATGCTTCATCCTCTTAAAGGTC	ACGTTGGATGCACAAAATATGAAACTTCC
rs1897083	ACGTTGGATGGTTCAACCTATCATTTTCTTC	ACGTTGGATGTAAGTCAATATGGATTAGAC
rs478588	ACGTTGGATGATCTCTTGAACCCAAGAGAT	ACGTTGGATGTGTTAAGGTTTATGTCTTG
rs664894	ACGTTGGATGCTTGAACCCAAGAGATGGAG	ACGTTGGATGTGGATTCTCTTCTGCTGCC

dbSNP rs#	Forward PCR primer	Reverse PCR primer
rs1594286	ACGTTGGATGCTTGAACCCAAGAGATGGAG	ACGTTGGATGTGAATTCTCTTTCTGCTGCC
rs7422168	ACGTTGGATGCTTGAACCCAAGAGATGGAG	ACGTTGGATGTGAATTCTCTTTCTGCTGCC
rs565202	ACGTTGGATGGCAAAGGCAATTCATGGAG	ACGTTGGATGCTCGCAGCCTATGTCTTGTT
rs1429974	ACGTTGGATGCTTCATTCTGGTCTGATTCA	ACGTTGGATGAAAGAATTCTATCAAGAAG
rs5829769	ACGTTGGATGGTTGGAGCAGATGTAAAGG	ACGTTGGATGGATCATGCTTCTGCCTTAAG
rs3056575	ACGTTGGATGGTTGGAGCAGATGTAAAGG	ACGTTGGATGGATCATGCTTCTGCCTTAAG
rs6708168	ACGTTGGATGATGGTTACAGTAGCACCTG	ACGTTGGATGTTTTTACGGCAGCCTGAGC
rs6756743	ACGTTGGATGTGGAATCGCAAGTGTAAGTG	ACGTTGGATGTTGCACATGTATCCCAGAAC
rs2195598	ACGTTGGATGATGGGCAAAGACTTCTTGAC	ACGTTGGATGTGCTGTCAGAAGCTCTTTAG
rs7567217	ACGTTGGATGCTCAAAACTCTTCTGGCCTC	ACGTTGGATGAACAGATGCTGGAGAGGATG
rs568938	ACGTTGGATGCTCCTCAGCTAAATATCCAG	ACGTTGGATGAAAGTGGCAAAGTACTTGGC
rs666416	ACGTTGGATGACCCCTTGAAACTGAGGTGG	ACGTTGGATGTCAGAAAGTCCTTAGGACTGC
rs6761300	ACGTTGGATGCCTACGAAGTAATTTTCTCC	ACGTTGGATGCTATATTGAATGACAAGAGG
rs5829770	ACGTTGGATGCACCTAACTGAGAATACACAG	ACGTTGGATGGCTGTAATTTCTTAGTGCC
rs1429973	ACGTTGGATGAAATATGGCTTGAACCCAGG	ACGTTGGATGTGGAGTGCAGTGGCAGCATCT
rs1429972	ACGTTGGATGCTTTCTTTGCTAACCCTGC	ACGTTGGATGCAGAATCTCTCTGAAAGCTG
rs6756284	ACGTTGGATGTGGGATTATAGGCATGAGCC	ACGTTGGATGTTTCAGCTTTCAGAGAGATTC
rs749988	ACGTTGGATGTTTTCTATTTGCATCTACTG	ACGTTGGATGGTGACAAAACAAACCAAAGTC
rs749987	ACGTTGGATGGTCTTCAAATATAGTATGGC	ACGTTGGATGATTTCCAGGGTTTGACTTTG
rs754524	ACGTTGGATGGACTTTCTGGGATTTCATC	ACGTTGGATGCTTCCACTCTAAGCCTTAAG
rs754523	ACGTTGGATGGTATTTGCAAAGTAGGTGAC	ACGTTGGATGTCTTGAAAGTGAAAGCCTCC
rs675430	ACGTTGGATGATGAGCATGACACAACAACC	ACGTTGGATGAGGTATCTTCAGAGACACAG
rs600012	ACGTTGGATGACTCCAGCCTGGGAGACAGA	ACGTTGGATGGCCTTGAACCTTACACTCAAG
rs614303	ACGTTGGATGCAAAACTCACATTCTTTGAC	ACGTTGGATGTTTAAATTCCTGCCATGCAC

TABLE 36

dbSNP rs#	Extend Primer	Term Mix
rs1318006	CCCTGACCTGTCACAGGG	ACG
rs1318005	ATGAGAGCCCACCTCCTGT	ACT
rs1318004	TGAGAGCCCACCTCCTGTA	ACG
rs1318003	ACTGGGAGACTCACAGGGA	ACT
rs4327259	GCCACTGGTCCAGCACAG	ACT
rs6756501	GCCACTTTCTCCTCCTGCT	ACG
rs6725189	AGAGAGGGCCGACTGCTG	CGT
rs4665709	GTCCCCACCCAAATCTCAC	ACT
rs4665710	GGCGGATTTCTCCTTTGGTG	CGT
rs4371387	GTTCCAGCCATGTAGGTTGT	ACT
rs952274	GGGTGACATGCATTGTGATTT	CGT
rs952275	CCTTCTGCTCAAAAACCTTTAC	ACT
rs1801695	ATTATTTTCTTCGTCGCAATGG	ACG
rs1042034	GAGATCAACACAATCTTCA	ACT

dbSNP rs#	Extend Primer	Term Mix
rs1801702	GATAAATCTTTCAACAGTTCC	ACT
rs1042031	TTGGTACGAGTTACTCAA	ACT
rs2678378	AGTCCTAGGAAGGCTTTAATTT	ACG
rs2678379	AGTCAGGAAATGACAGATAGG	ACT
rs1800479	GGTATGGAGATGAAGAAAATCA	ACT
rs1801701	AAAGACCCAGAATGAATC	ACG
rs4362589	GGGCACTTCCAAAATTGATGAT	CGT
rs5742904	CCTGCAGCTTCACTGAAGAC	ACG
rs1799812	GGTGCCCTCTAATTTGTACTG	ACG
rs2163204	GCTGCGATACCTGCTTCGT	ACT
rs676210	AAGTTCCTGACCTTCACATAC	ACG
rs1042006	CTGATGATCTTTACTTTTCATTTT	ACT
rs1801696	GAACCTTACTGACTTTGCA	ACT
rs693	GGCCAAATTCGAGAGAC	ACG
rs1041974	GTTGGATTTATTGATGATGCTGT	ACT
rs1041968	TTTGACATGCTCAAGAAC	ACT
rs568413	TGGCGTAGAGACCCATCA	ACT
rs2854726	AGCCTGTAGTCAATAACGCC	CGT
rs2854725	AGCTTTGTGTAAGTGGTAAC	ACT
rs2000998	TATCTGGTTTTGATCACCACAT	CGT
rs2000997	CAGGATTAAACAGAAGTTCCAA	CGT
rs497166	AGTGCTGGGATTACAGGTGT	ACT
rs562956	CGGCTTCTCCTCTTATTTCTG	CGT
rs7589300	AAGGTCCCTGACCTTTGAAC	ACT
rs3791980	GGAAAATTAATATTTTCCCCC	CGT
rs3791981	GAGAATGAAGAAACAATAGCTC	ACG
rs1801700	GACTCGTGGAAGAAGTTGGT	ACT
rs679899	AAGTTGAGATTCTTTCAGA	ACT
rs1041952	CAGAACTCAAGTCTTCAATCCT	ACT
rs6727706	TCCCTAGTGTATGTTTTGTCA	ACT
rs6719207	TGTAACCATGTCAACAGTAGC	CGT
rs1469513	CAAGCCTCTGGCCTTTGAAG	ACT
rs1800478	CATACACGGTATCCTATGGAG	ACT
rs550619	GTGGCCAGGACTCCTCAAT	ACT
rs6752026	GGAAGCAGGTTTTCTTTAC	ACG
rs579826	TGAGCCACCAGGTCCAGC	ACG
rs597331	CTCTCAGAAAGTTCCCAACAC	ACT
rs1367116	TTAAAGGAACCTAACTAGGGAA	ACT
rs1367117	AGCCATACACCTCTTTCAGG	ACT
rs1800480	GGCACTCAGCCCCGCAG	ACT
rs1800481	TCTCAGACCCTGAGGCGC	ACG
rs934197	CTGCATCCCCCTTCTCTCT	ACG
rs1625764	CATCGAGCGCTGGCTGAAG	ACG

dbSNP rs#	Extend Primer	Term Mix
rs1625714	TCCAGCTGGGCAGAGGCA	ACT
rs1560357	CCACTACCGCTGATTCCCT	CGT
rs617314	GTAGCTTGTTACATCTGGGG	ACT
rs547186	GGGAAGACTTGCCAAAGACC	CGT
rs589566	TCTGAGTTTAGTGCTGTTTAC	ACT
rs588245	AGCCTATCTCGTTTCTGCCT	ACT
rs585967	CTATGAAGTCTAACTGGGCTG	ACT
rs7562777	ATGGTGCCTCGTGCCTGTA	ACT
rs7575840	TCACAGGGAAAGCCAGGAAT	ACT
rs7567653	ACTTCATTAATAACATCGCCGT	ACT
rs6548010	GGTTTTTGGTATACACATATTC	ACT
rs6548011	AAGGATAGAAAAAATATAGTCCC	ACT
rs934198	AGGAGGATGAGTGGGGAGA	ACT
rs634292	CTTGTTTATGGCACAGAAGATG	ACT
rs1003177	CACCATTTATGCAGGGCTAG	ACT
rs6726115	CTGGTACTTGGTTAATAGTCC	ACT
rs481069	CAGGACCCCAGCCCCCA	ACT
rs1367115	TGGATTAGTGAATGGGAGGG	ACT
rs666126	GCAGGATTCATCTCTCCATATA	ACG
rs7566030	TGCCTGCCCCAACCCTCT	ACT
rs7590135	CACCAGGCTGTTTTAGCAGC	ACG
rs6718513	AAGAACAAAAGAGGATTGGGA	ACT
rs515135	ACAGCCAAAATGGAACCAAAG	ACT
rs1367114	TTGCAGGTCACTTTTTTAAAGTT	ACT
rs563290	AACACAGAAATGCAGATATCTC	ACG
rs562338	CATTGTCTTGACAGATGAATGC	ACT
rs581411	TGATAGAGACAGTTATCAATTTT	ACT
rs580889	TCTCCGGCTGGGCCGTC	ACT
rs548145	AGAAACAATGACAGAATACTAAG	ACT
rs668948	GGCATGCTGTCTCCTCTGC	ACT
rs594677	GTATTGAACAGGACTGAGTAAT	ACG
rs571468	GAAGAGAAGGCTGGCGCC	CGT
rs4665492	CCTATAGATAAGACTTTTATTCCA	ACT
rs622236	GTGAATGAATGAATGAATGAACC	CGT
rs541041	CTATTCATGTTTCAGGGCCCA	ACG
rs540156	TACGAGTATATGTATACATTTGC	ACT
rs1367113	GGCTAGATAGGGAAGTGGG	ACT
rs1897084	TCTTAAAAGGTCTTTTGCAAAGA	ACT
rs1897083	TCTATATTTCTTTTGGGAAGTTT	ACT
rs478588	CTGGGCAGCAGAAAGAGAAT	ACG
rs664894	GCCAAGATCATGCCACTGC	CGT
rs1594286	ATGGAGGTTGCAGTGAGCC	ACT
rs7422168	GATGGAGGTTGCAGTGAGC	ACT

dbSNP rs#	Extend Primer	Term Mix
rs565202	CAGGAACAATTGGAAGTCTACA	ACG
rs1429974	CTGGTCTGATTTCAGTTGCC	ACT
rs5829769	GAGGATATATTCCAGGAGATATA	CGT
rs3056575	CAGAGGATATATTCCAGGAGA	CGT
rs6708168	CCCTGCTTCTCAGTACCAAA	CGT
rs6756743	CGCAAGTGTAAAGTGATCAAAG	ACG
rs2195598	ACTAAAACACCAAAAGCAATGG	ACG
rs7567217	ACTCTTCTGGCCTCATCTAC	ACT
rs568938	CCTCACACAAAACACCAGAAC	ACT
rs666416	GCCTGTCCCACTGGGCC	ACG
rs6761300	GGAATTCTTCAATAATGACAACA	ACT
rs5829770	CTTGATAACATGTACCAAAAAAAAA	CGT
rs1429973	CTTGAACCCAGGAGGCAGA	ACT
rs1429972	GCTAACCACTGCAGCTCCT	ACG
rs6756284	GGCATGAGCCACCGCGC	ACG
rs749988	TCTATTTGCATCTACTGAATTTTT	ACG
rs749987	CGAATAAGGAGCTATCTGTGA	ACG
rs754524	TAGAAAACAAGCTATACATTCATA	ACT
rs754523	TGCAAAGTAGGTGACAATTGC	ACG
rs675430	GTGAAAAATGAACAGATTGTCC	ACT
rs600012	CTGGGAGACAGAGCGAGATT	ACG
rs614303	CTTTGACAATACATGAGCCCT	ACG

Genetic Analysis

[0269] Allelotyping results from the discovery cohort are shown for cases and controls in Table 37. The allele frequency for the A2 allele is noted in the fifth and sixth columns for osteoarthritis case pools and control pools, respectively, where “AF” is allele frequency. The allele frequency for the A1 allele can be easily calculated by subtracting the A2 allele frequency from 1 (A1 AF = 1-A2 AF). For example, the SNP rs1318006 has the following case and control allele frequencies: case A1 (C) = 0.494; case A2 (T) = 0.506; control A1 (C) = 0.460; and control A2 (T) = 0.540, where the nucleotide is provided in paranthesis. Some SNPs are labeled “untyped” because of failed assays.

TABLE 37

dbSNP rs#	Position in SEQ ID NO: 1	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs1318006	238	21188688	C/T	0.506	0.540	0.326
rs1318005	294	21188744	C/T	0.044	0.034	0.643
rs1318004	295	21188745	A/G			
rs1318003	347	21188797	A/C			
rs4327259	1425	21189875	A/C	0.962	0.965	0.865

dbSNP rs#	Position in SEQ ID NO: 1	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs6756501	4891	21193341	C/T	0.195	0.141	0.061
rs6725189	5087	21193537	G/T	0.317	0.250	0.036
rs4665709	7041	21195491	A/G	0.683	0.757	0.014
rs4665710	7121	21195571	A/C	0.206	0.209	0.926
rs4371387	7219	21195669	A/G	0.579	0.688	~0.0001
rs952274	7443	21195893	G/T	0.163	0.123	0.158
rs952275	7485	21195935	G/T	0.234	0.319	0.013
rs1801695	10939	21199389	A/G	0.047	0.071	0.319
rs1042034	11367	21199817	A/G	0.191	0.182	0.743
rs1801702	11571	21200021	C/G			
rs1042031	11839	21200289	A/G	0.686	0.785	0.001
rs2678378	12551	21201001	A/G			
rs2678379	12646	21201096	A/G	0.693	0.714	0.466
rs1800479	13469	21201919	G/C	0.144	0.130	0.687
rs1801701	14913	21203363	A/G	0.090	0.116	0.314
rs4362589	15205	21203655	G/T			
rs5742904	15246	21203696	A/G			
rs1799812	15695	21204145	G/A			
rs2163204	17473	21205923	G/T			
rs676210	17610	21206060	A/G	0.186	0.177	0.758
rs1042006	17828	21206278	A/C			
rs1801696	18130	21206580	A/G			
rs693	18281	21206731	C/T	0.494	0.537	0.208
rs1041974	18623	21207073	C/G			
rs1041968	18890	21207340	C/T			
rs568413	21561	21210011	C/T			
rs2854726	23100	21211550	A/T			
rs2854725	23872	21212322	A/C			
rs2000998	24581	21213031	A/T			
rs2000997	24582	21213032	A/T			
rs497166	24983	21213433	C/T			
rs562956	27540	21215990	A/T			
rs7589300	30846	21219296	C/T			
rs3791980	31415	21219865	G/T			
rs3791981	31453	21219903	A/G	0.964	0.968	0.849
rs1801700	31899	21220349	T/C	0.832	0.897	0.008
rs679899	37000	21225450	A/G	0.378	0.474	0.004
rs1041952	38681	21227131	C/G			
rs6727706	39287	21227737	C/T			
rs6719207	42951	21231401	A/T			
rs1469513	45648	21234098	C/T	0.477	0.534	0.079
rs1800478	46222	21234672	C/T			
rs550619	46687	21235137	A/G	0.053	0.062	0.656
rs6752026	47020	21235470	A/G			
rs579826	47593	21236043	C/T	0.069	0.063	0.817
rs597331	48513	21236963	C/T	0.435	0.512	0.014
rs1367116	49723	21238173	A/G			
rs1367117	49986	21238436	A/G	0.431	0.367	0.049
rs1800480	53018	21241468	C/G	0.978	NA	NA
rs1800481	53296	21241746	C/T	0.100	0.082	0.487
rs934197	53547	21241997	A/G	0.338	0.398	0.075
rs1625764	53899	21242349	C/T			
rs1625714	53916	21242366	G/T			
rs1560357	53933	21242383	A/C			
rs617314	54305	21242755	G/T	0.977	0.971	0.741
rs547186	55327	21243777	A/T	0.468	0.490	0.528

dbSNP rs#	Position in SEQ ID NO: 1	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs589566	55895	21244345	C/T	0.386	0.377	0.780
rs588245	56143	21244593	C/T	0.425	0.398	0.397
rs585967	56640	21245090	G/T	0.724	0.781	0.046
rs7562777	58486	21246936	A/G			
rs7575840	59576	21248026	G/T	0.436	0.408	0.422
rs7567653	63048	21251498	A/G	0.918	0.910	0.739
rs6548010	64008	21252458	A/G	0.293	0.345	0.081
rs6548011	64018	21252468	C/T	0.530	0.482	0.135
rs934198	64859	21253309	A/C	0.526	0.484	0.225
rs634292	65995	21254445	G/T	0.456	0.492	0.256
rs1003177	66905	21255355	A/G			
rs6726115	67183	21255633	A/G	0.293	0.342	0.119
rs481069	67942	21256392	C/T	0.138	0.104	0.167
rs1367115	68101	21256551	A/G	0.421	0.408	0.693
rs666126	68521	21256971	A/G	0.500	0.530	0.388
rs7566030	68664	21257114	C/G	0.397	0.416	0.536
rs7590135	68988	21257438	A/G	0.268	0.324	0.082
rs6718513	69178	21257628	C/G			
rs515135	72143	21260593	A/G	0.726	0.747	0.455
rs1367114	74183	21262633	C/G			
rs563290	74312	21262762	C/T	0.667	0.690	0.516
rs562338	74407	21262857	C/T	0.482	0.578	0.006
rs581411	75518	21263968	A/G	0.162	0.157	0.839
rs580889	76153	21264603	A/G	0.127	0.111	0.487
rs548145	77398	21265848	A/G	0.709	0.765	0.049
rs668948	77615	21266065	A/G	0.133	0.127	0.805
rs594677	79092	21267542	C/T			
rs571468	80000	21268450	G/T	0.455	0.502	0.169
rs4665492	80125	21268575	A/C	0.274	0.327	0.088
rs622236	80595	21269045	G/T			
rs541041	81061	21269511	C/T	0.779	0.791	0.694
rs540156	81151	21269601	A/G	0.237	0.277	0.237
rs1367113	81918	21270368	C/T	0.394	0.366	0.370
rs1897084	83072	21271522	C/T			
rs1897083	83137	21271587	C/T	0.279	0.326	0.139
rs478588	83235	21271685	C/T			
rs664894	83263	21271713	A/T	0.319	0.343	0.467
rs1594286	83279	21271729	A/G			
rs7422168	83280	21271730	C/G			
rs565202	83533	21271983	C/T	0.483	0.514	0.373
rs1429974	86856	21275306	G/T	0.583	0.535	0.189
rs5829769	87186	21275636	-/TATA			
rs3056575	87189	21275639	-/ATAT			
rs6708168	87727	21276177	A/T	0.610	0.563	0.163
rs6756743	87978	21276428	C/T	0.051	0.051	0.978
rs2195598	89129	21277579	A/G			
rs7567217	89556	21278006	C/T	0.100	0.087	0.547
rs568938	89702	21278152	A/G	0.177	0.150	0.304
rs666416	90233	21278683	A/G	0.421	0.364	0.093
rs6761300	93060	21281510	A/G	0.271	0.348	0.012
rs5829770	94779	21283229	-/T	0.036	0.037	0.971
rs1429973	95367	21283817	A/G			
rs1429972	95844	21284294	A/G	0.422	0.443	0.533
rs6756284	95942	21284392	A/G	0.155	0.114	0.133
rs749988	96884	21285334	C/T			
rs749987	96938	21285388	A/G			

dbSNP rs#	Position in SEQ ID NO: 1	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs754524	97627	21286077	A/C	0.248	0.306	0.044
rs754523	97777	21286227	C/T	0.567	0.512	0.113
rs675430	97871	21286321	A/C	0.352	0.345	0.812
rs600012	98746	21287196	A/G			
rs614303	99663	21288113	A/G	0.722	0.730	0.805

[0270] Allelotyping results were considered particularly significant with a calculated p-value of less than or equal to 0.05 for allelotype results. These values are indicated in bold. The allelotyping p-values were plotted in Figure 1A for the discovery cohort. The position of each SNP on the chromosome is presented on the x-axis. The y-axis gives the negative logarithm (base 10) of the p-value comparing the estimated allele in the case group to that of the control group. The minor allele frequency of the control group for each SNP designated by an X or other symbol on the graphs in Figure 1A can be determined by consulting Table 37. For example, the left-most X on the left graph is at position 21188688. By proceeding down the Table from top to bottom and across the graphs from left to right the allele frequency associated with each symbol shown can be determined.

[0271] To aid the interpretation, multiple lines have been added to the graph. The broken horizontal lines are drawn at two common significance levels, 0.05 and 0.01. The vertical broken lines are drawn every 20kb to assist in the interpretation of distances between SNPs. Two other lines are drawn to expose linear trends in the association of SNPs to the disease. The light gray line (or generally bottom-most curve) is a nonlinear smoother through the data points on the graph using a local polynomial regression method (W.S. Cleveland, E. Grosse and W.M. Shyu (1992) Local regression models. Chapter 8 of Statistical Models in S eds J.M. Chambers and T.J. Hastie, Wadsworth & Brooks/Cole.). The black line provides a local test for excess statistical significance to identify regions of association. This was created by use of a 10kb sliding window with 1kb step sizes. Within each window, a chi-square goodness of fit test was applied to compare the proportion of SNPs that were significant at a test wise level of 0.01, to the proportion that would be expected by chance alone (0.05 for the methods used here). Resulting p-values that were less than 10^{-8} were truncated at that value.

[0272] Finally, the exons and introns of the genes in the covered region are plotted below each graph at the appropriate chromosomal positions. The gene boundary is indicated by the broken horizontal line. The exon positions are shown as thick, unbroken bars. An arrow is place at the 3' end of each gene to show the direction of transcription.

Example 9

BVES Proximal SNPs

[0273] It has been discovered that rs 1018810 is associated with occurrence of osteoarthritis in subjects. BVES was identified as a blood vessel epicardial substance. Sequence analysis predicted 3 transmembrane helices with an extracellular C terminus. Northern blot analysis revealed that expression of an approximately 5.5-kb BVES transcript is restricted to skeletal muscle and adult and fetal heart. BVES is highly expressed in osteoarthritic cartilage according to EST database analysis, and may play a role in chondrocyte and/or bone cell development. BVES biological activity may be modulated by addition of an antibody, a recombinant binding partner, a binding agent, or a recombinant BVES protein or functional fragment thereof

[0274] One hundred fifty-four additional allelic variants proximal to rs 1018810 were identified and subsequently allelotyped in osteoarthritis case and control sample sets as described in Examples 1 and 2. The polymorphic variants are set forth in Table 38. The chromosome positions provided in column four of Table 38 are based on Genome "Build 34" of NCBI's GenBank.

TABLE 38

dbSNP rs#	Chromosome	Position in SEQ ID NO: 4	Chromosome Position	Allele Variants
rs2400080	6	241	105557091	A/G
rs6930209	6	801	105557651	A/G
rs221628	6	899	105557749	A/G
rs221629	6	2091	105558941	C/G
rs221630	6	2290	105559140	C/T
rs221631	6	2440	105559290	A/G
rs1149284	6	4959	105561809	G/T
rs221633	6	7914	105564764	C/G
rs423366	6	7969	105564819	A/G
rs436460	6	7972	105564822	C/T
rs2211010	6	10831	105567681	C/T
rs379908	6	12399	105569249	C/T
rs1149285	6	13841	105570691	C/T
rs7341194	6	14461	105571311	C/T
rs715153	6	14680	105571530	C/T
rs221634	6	16808	105573658	A/T
rs7757307	6	18231	105575081	C/T
rs221635	6	18394	105575244	C/T
rs4145418	6	18505	105575355	G/T
rs221636	6	18684	105575534	A/T
rs3185958	6	19257	105576107	C/T
rs4946654	6	20263	105577113	A/T
rs221637	6	20656	105577506	A/C

dbSNP rs#	Chromosome	Position in SEQ ID NO: 4	Chromosome Position	Allele Variants
rs221638	6	21499	105578349	A/G
rs221639	6	21563	105578413	A/C
rs643545	6	21612	105578462	C/G
rs221640	6	21834	105578684	C/T
rs3957696	6	22406	105579256	A/T
rs3995554	6	22408	105579258	A/T
rs7453502	6	22685	105579535	A/T
rs1190471	6	23303	105580153	C/T
rs221641	6	23306	105580156	C/G
rs221642	6	25139	105581989	A/G
rs1190472	6	25211	105582061	C/T
rs1190473	6	25364	105582214	A/G
rs186404	6	25381	105582231	A/C
rs221643	6	25414	105582264	A/T
rs221644	6	25835	105582685	C/T
rs1203475	6	26214	105583064	A/G
rs221645	6	27224	105584074	A/G
rs170277	6	27526	105584376	A/G
rs221646	6	27934	105584784	C/T
rs221647	6	28550	105585400	C/T
rs221648	6	29015	105585865	A/G
rs221649	6	29879	105586729	G/T
rs221650	6	29979	105586829	A/G
rs1149287	6	30030	105586880	A/G
rs221651	6	30585	105587435	C/T
rs7762591	6	31753	105588603	C/G
rs7748555	6	31934	105588784	C/T
rs5878833	6	33227	105590077	-/T
rs5878834	6	33228	105590078	-/T
rs221652	6	35172	105592022	C/T
rs221653	6	36901	105593751	A/G
rs221654	6	36921	105593771	A/G
rs221655	6	36932	105593782	A/G
rs221656	6	37061	105593911	C/T
rs221657	6	37570	105594420	C/T
rs221658	6	38745	105595595	G/T
rs110065	6	38970	105595820	A/T
rs221659	6	39725	105596575	C/T
rs221660	6	40070	105596920	A/C
rs7742821	6	40460	105597310	C/G
rs221662	6	41470	105598320	A/G
rs7748426	6	41562	105598412	A/G
rs6911494	6	41956	105598806	A/G
rs6939846	6	42047	105598897	A/T
rs368471	6	42280	105599130	A/G
rs430190	6	42358	105599208	A/G

dbSNP rs#	Chromosome	Position in SEQ ID NO: 4	Chromosome Position	Allele Variants
rs455114	6	42629	105599479	C/G
rs405956	6	43075	105599925	C/T
rs5878835	6	43387	105600237	-/A
rs1473814	6	43393	105600243	G/T
rs423272	6	43438	105600288	C/T
rs413806	6	44115	105600965	A/G
rs4946655	6	44537	105601387	A/G
rs6915632	6	45642	105602492	A/G
rs2095723	6	46629	105603479	A/G
rs7450078	6	47496	105604346	A/G
rs7453071	6	47515	105604365	A/C
rs1018810	6	48329	105605179	A/G
rs7450944	6	48862	105605712	C/G
rs7748657	6	48908	105605758	A/G
rs1013137	6	49038	105605888	C/T
rs5878836	6	49080	105605930	-/T
rs1981480	6	50204	105607054	A/T
rs1981479	6	50404	105607254	A/G
rs3035187	6	50426	105607276	-/TTA
rs7453993	6	50531	105607381	C/T
rs2001119	6	50840	105607690	C/T
rs2001118	6	50964	105607814	C/T
rs2001117	6	50971	105607821	C/T
rs6940433	6	51378	105608228	C/T
rs1318746	6	52610	105609460	A/C
rs763099	6	53906	105610756	A/T
rs5878837	6	53951	105610801	-/C
rs964731	6	54111	105610961	A/C
rs964730	6	54149	105610999	G/T
rs6921869	6	55563	105612413	C/G
rs3945029	6	55999	105612849	C/T
rs4945715	6	58415	105615265	C/G
rs7775252	6	58961	105615811	C/G
rs7742098	6	60447	105617297	C/T
rs3757289	6	61377	105618227	A/G
rs6905458	6	61528	105618378	A/G
rs3757290	6	61606	105618456	C/G
rs2275289	6	62140	105618990	A/G
rs4945716	6	62461	105619311	C/T
rs6922638	6	63826	105620676	C/T
rs7739572	6	64950	105621800	G/T
rs6901187	6	65076	105621926	G/T
rs4946656	6	66121	105622971	C/T
rs1338020	6	66406	105623256	C/T
rs7771472	6	67051	105623901	A/C
rs6926260	6	68860	105625710	C/T

dbSNP rs#	Chromosome	Position in SEQ ID NO: 4	Chromosome Position	Allele Variants
rs6926627	6	69014	105625864	C/T
rs4946657	6	70796	105627646	C/T
rs6571218	6	72325	105629175	G/T
rs7449944	6	73414	105630264	A/C
rs952175	6	75258	105632108	C/G
rs1890228	6	76347	105633197	A/G
rs1933237	6	76839	105633689	A/C
rs1338019	6	77358	105634208	A/G
rs7453127	6	77822	105634672	A/G
rs7381551	6	77946	105634796	G/T
rs6571219	6	80002	105636852	A/G
rs6571220	6	80024	105636874	A/G
rs2185017	6	80285	105637135	A/G
rs1591720	6	80397	105637247	C/G
rs6925046	6	82075	105638925	C/T
rs6940423	6	82153	105639003	A/G
rs1190274	6	83981	105640831	A/G
rs1190276	6	84184	105641034	A/G
rs1591719	6	85089	105641939	C/T
rs1933236	6	85288	105642138	A/G
rs6905202	6	85330	105642180	C/T
rs1209150	6	85581	105642431	A/T
rs1190277	6	85642	105642492	A/G
rs6926278	6	86433	105643283	A/G
rs1190278	6	86904	105643754	A/G
rs4626463	6	88391	105645241	A/G
rs6924620	6	89042	105645892	C/T
rs1190280	6	90828	105647678	G/T
rs4557552	6	92676	105649526	C/T
rs6932711	6	92881	105649731	C/T
rs1686140	6	94227	105651077	G/T
rs1190281	6	94585	105651435	A/G
rs2308162	6	94616	105651466	-/ATAA
rs1190282	6	94712	105651562	C/G
rs1765907	6	94738	105651588	A/G
rs5878838	6	95253	105652103	-/G
rs1190283	6	95522	105652372	A/G
rs1190284	6	95869	105652719	G/T
rs1190285	6	97856	105654706	C/T

Assay for Verifying and Allelotyping SNPs

[0275] The methods used to verify and allelotype the 154 proximal SNPs of Table 38 are the same methods described in Examples 1 and 2 herein. The primers and probes used in these assays are provided in Table 39 and Table 40, respectively.

TABLE 39

dbSNP rs#	Forward PCR primer	Reverse PCR primer
rs2400080	ACGTTGGATGGTGGCCAGCAAGTGATGATA	ACGTTGGATGACAGAGCAAGACTCCATCTC
rs6930209	ACGTTGGATGGCTCTGTGGTGCATATTTAC	ACGTTGGATGGGTTCTCTCACTTAAGTGTG
rs221628	ACGTTGGATGAGTGAGAGAACCAAAATGTTG	ACGTTGGATGCCAGTTTTGGCTTCATTTGC
rs221629	ACGTTGGATGTCTGTCCATTTCTCCCTCTG	ACGTTGGATGGCTGATTCTTGGCAAAAGGC
rs221630	ACGTTGGATGTCCTTCTCATTGCTGTGTAG	ACGTTGGATGTCATGTGCAAGAGCCAAAAG
rs221631	ACGTTGGATGCACTGGCCCTCTATAAATGC	ACGTTGGATGCCAGCCCCCTGCATTATTAT
rs1149284	ACGTTGGATGGATGAGAAATTAAGTAGACAC	ACGTTGGATGGTCCATTTGGTTTTCATTTG
rs221633	ACGTTGGATGCTTAACAATTTGTCTTGGAG	ACGTTGGATGAGCCACATATACCAAAAAAC
rs423366	ACGTTGGATGAGCCACATATACCAAAAAAC	ACGTTGGATGGAGATCTTTCATGTCAATAC
rs436460	ACGTTGGATGAGCCACATATACCAAAAAAC	ACGTTGGATGGAGATCTTTCATGTCAATAC
rs2211010	ACGTTGGATGTTTTTTGAGACAGAGTCTCG	ACGTTGGATGTTTGCAGTGAGCTGAGATTG
rs379908	ACGTTGGATGTGAGTGGGCAAAATGGTTCC	ACGTTGGATGCTCTCCTGCAGACACATCAA
rs1149285	ACGTTGGATGCCAAATACATTTATGACTCC	ACGTTGGATGGAGAGAGATTCCATCTCAAA
rs7341194	ACGTTGGATGCTGTAGAAACCAGCTAAACTG	ACGTTGGATGCTGACTAGACTCTGACTTTC
rs715153	ACGTTGGATGTTTTGTTGAATATTCGCTGC	ACGTTGGATGCTTCCATATAGAAAGGATTCC
rs221634	ACGTTGGATGTGCCATAACATCTAGAGCC	ACGTTGGATGTTGGTCTGTTAGGTTTCGG
rs7757307	ACGTTGGATGTGCTTAAGTTGAACAGTGCC	ACGTTGGATGGCAAAGTCTCAAACATTTCC
rs221635	ACGTTGGATGGGCAGCACAGACAGTAAATG	ACGTTGGATGTGCAGGTATTGCTAGGC
rs4145418	ACGTTGGATGTGCATTGCCAGTCTCTTAGC	ACGTTGGATGGGCCTTCTAGTGAAGACTAG
rs221636		
rs3185958	ACGTTGGATGGACACAGATCATACAACCAC	ACGTTGGATGAGCATCAAAGTCTGTCTTAC
rs4946654	ACGTTGGATGATGTAGTCAGAAGAGTGGTC	ACGTTGGATGGGTAAGTATATAAATTTGCC
rs221637	ACGTTGGATGCAATCGTAGCTTACTGTGGG	ACGTTGGATGCTGTAGTCCAGCTACTCAAG
rs221638	ACGTTGGATGCACACCTGGCTGAAATCTTA	ACGTTGGATGTGGTTATTTCTAGGCCGATGG
rs221639	ACGTTGGATGCCCGCATGTGTATGTATCTC	ACGTTGGATGCCCATCGCCTAGAAATAACC
rs643545	ACGTTGGATGAAATCACCCGCATGTGTAT	ACGTTGGATGCGCCTAGAAATAACCATTAGC
rs221640	ACGTTGGATGTAATCCAGCACTTTGGGAG	ACGTTGGATGTTTACCAGTGTAGCCAGGC
rs3957696	ACGTTGGATGAACCAAGTATGTTGCCCTTTC	ACGTTGGATGCCAGGCAGTCCAAATTAATTC
rs3995554	ACGTTGGATGAACCAAGTATGTTGCCCTTTC	ACGTTGGATGCCAGGCAGTCCAAATTAATTC
rs7453502	ACGTTGGATGCTCCAAGGTTGGAGTTTGTG	ACGTTGGATGTTTCTGAGCTCCTCAGCATC
rs1190471	ACGTTGGATGATATGTGGCCCGATGATCTC	ACGTTGGATGCCTCCCAAGTGCTAGGATT
rs221641	ACGTTGGATGCCTCCCAAGTGCTAGGATT	ACGTTGGATGATATGTGGCCCGATGATCTC
rs221642	ACGTTGGATGTCTTCCACCATGATTGTGAG	ACGTTGGATGAGACATACCTGAGACTGGAC
rs1190472	ACGTTGGATGTGTCAGTCTCAGGTATGTC	ACGTTGGATGGCCAGCTAAGGTTTTGTAG
rs1190473	ACGTTGGATGTTGATCACACCACTGCACTC	ACGTTGGATGCCCCAATGAAGAAGTCTTGC
rs186404	ACGTTGGATGTTGATCACACCACTGCACTC	ACGTTGGATGCCCCAATGAAGAAGTCTTGC
rs221643	ACGTTGGATGCCCCAATGAAGAAGTCTTGC	ACGTTGGATGGAGACACAGTGAGACTGTCA

dbSNP rs#	Forward PCR primer	Reverse PCR primer
rs221644	ACGTTGGATGGTGTCTTTCTAGCTAGCTC	ACGTTGGATGTTACAGATGGGTTTCAGGGAG
rs1203475	ACGTTGGATGTAATCCCAGCTACTTGGGAG	ACGTTGGATGACAATCTCGGCTCACTGCAA
rs221645	ACGTTGGATGTGTTTTTCATCTGCCCAATG	ACGTTGGATGGCTGCTGTAAAGGACCACAT
rs170277	ACGTTGGATGACAAGGAAGTTCTGAACCTC	ACGTTGGATGTTTTGGATCAAGAGGTGACC
rs221646	ACGTTGGATGAATTGGCTCTTCTCTCTGCC	ACGTTGGATGTTACAGCAGAAATGGCTGGA
rs221647	ACGTTGGATGTTCCCAGCTCCTTTCTTAG	ACGTTGGATGTTCTTAAGAAAATGCCCTC
rs221648	ACGTTGGATGATCATGCCACTGCACTCCAG	ACGTTGGATGTTAGGTCTCCAGGACGACAG
rs221649	ACGTTGGATGGACAGGATGAAGAAGAAGGC	ACGTTGGATGTCTTGCTATTTCGCCAAGGAC
rs221650	ACGTTGGATGTAATATCCAGGATCCAGCTG	ACGTTGGATGTTGAACCCCTGAACCTCAAGC
rs1149287	ACGTTGGATGATGGAGGTCTCACCATTGTC	ACGTTGGATGTAGCACTTTGGGAGGCCAAG
rs221651	ACGTTGGATGGGAGGATCACTTGAATCCAG	ACGTTGGATGAGACAGGTTCTTGCTCTGTT
rs7762591	ACGTTGGATGATCTCTGCTCACTGCAGCTT	ACGTTGGATGAAATTAGCCAGGTGTGGTGG
rs7748555	ACGTTGGATGTTGGGATTACAGGTGTGAGC	ACGTTGGATGCCCACTGCTTCACTTGACTA
rs5878833	ACGTTGGATGACACTGTCTACACTGCCTTC	ACGTTGGATGACCTGACTTCAAAGGTCTTG
rs5878834	ACGTTGGATGACACTGTCTACACTGCCTTC	ACGTTGGATGACCTGACTTCAAAGGTCTTG
rs221652	ACGTTGGATGTACTTTCTACTCAGGGAAGG	ACGTTGGATGAGTTTACACGCGCATAAGAC
rs221653	ACGTTGGATGGTTTCACTGTGTTAGCCAGG	ACGTTGGATGTAATCCCAGCACTCTGGGAG
rs221654	ACGTTGGATGGAGATCAAGACCATCCTGGC	ACGTTGGATGAGTAGCTGGGACTACAGGCA
rs221655	ACGTTGGATGGTCAGGAGATCAAGACCATC	ACGTTGGATGCCGCGCCCAGCTAATTTTTT
rs221656	ACGTTGGATGAGATGGAGTTTCACTCTGTC	ACGTTGGATGAATCCAGGAGGTGGAGTTTG
rs221657	ACGTTGGATGAGAACTCTTCCATCCTTGAC	ACGTTGGATGTTCTGCTTTAGTGCATCCAG
rs221658	ACGTTGGATGCCAGCTGAGTTCAGCATTTG	ACGTTGGATGACACCCATATCTTCGCTACC
rs110065	ACGTTGGATGTGACATGCTCATAGCCCTTG	ACGTTGGATGAGATCAGCTGTCATTCACTG
rs221659	ACGTTGGATGCGAAACACAACCTCTACTTC	ACGTTGGATGCAGGTAAGGAAATTAAGGCAC
rs221660	ACGTTGGATGAATATGATGGAACCAGGGC	ACGTTGGATGCTTAGCTCTCTTGAGTGTG
rs7742821	ACGTTGGATGAGCTCTTGGGAAGTTCTCAC	ACGTTGGATGCCCACTCTCTCACCTATAC
rs221662	ACGTTGGATGGACAATGGGTTAAATGTTGGG	ACGTTGGATGAAGTGCTTTGAGTTTCTGAG
rs7748426	ACGTTGGATGATTCACCCCTCACCACATCTG	ACGTTGGATGCCACCCCTCTCTGTTTTCTT
rs6911494	ACGTTGGATGTCAATGGTACAGAAGGCCAG	ACGTTGGATGAACCCCTCGCTTGAATTAG
rs6939846	ACGTTGGATGTCCTCAAAGCTGGGCTTTCT	ACGTTGGATGAGACAAAAGGATCACCTGCC
rs368471	ACGTTGGATGCCCCCTAATACATCCAAAACC	ACGTTGGATGACCAGGCCAAACCTGTAGAAG
rs430190	ACGTTGGATGTCTCTGGAAGATAGTTGGGC	ACGTTGGATGACTTCTACAGGTTTGCCTGG
rs455114	ACGTTGGATGCCCAGAAAATTGATTCTTAG	ACGTTGGATGACAGAAGTCTTTTCTGATC
rs405956	ACGTTGGATGAAACTCCAAGTCAAGGACCC	ACGTTGGATGAAAGGTGTCCACTGTTTCGC
rs5878835	ACGTTGGATGCTGTCTTCCAGAGTCTTGAG	ACGTTGGATGTACATCCACTATGTACCCAC
rs1473814	ACGTTGGATGGTTAAAGAACCACAGAAGGC	ACGTTGGATGTACATCCACTATGTACCCAC
rs423272	ACGTTGGATGCACAGAAGGCCTTAAAAACC	ACGTTGGATGTCACGTTGCATTCTGTATC
rs413806	ACGTTGGATGCTGACAGATTTACATCGTG	ACGTTGGATGGTTCCAGAGGATGAACAAAC
rs4946655	ACGTTGGATGCTAAAGAGTAGCTTTGGCTTG	ACGTTGGATGTTTTGTACGCTTTGCCTGAG
rs6915632	ACGTTGGATGGTCGTGATCTTGACTCACTG	ACGTTGGATGGCCTGTAAATCCCAGTTACTC
rs2095723	ACGTTGGATGTGTGCTCTCTCATGCCAGTA	ACGTTGGATGCTGTATAAAATACCTTCAGG
rs7450078	ACGTTGGATGGCCATCACCTCCAGATAATT	ACGTTGGATGAAGGCAGGAGGATCTCTTGA
rs7453071	ACGTTGGATGAATCCCAGCACTTTGGGAGG	ACGTTGGATGTATGTTGCCAGGCTCGTCT
rs1018810	ACGTTGGATGTGCTGCTCCCATTCTCATG	ACGTTGGATGAAGGAGTAGAGACCTTGCTG
rs7450944	ACGTTGGATGATTACGCCACTACACCTCAG	ACGTTGGATGGTTGTTCTACAGGACAAACC
rs7748657	ACGTTGGATGAGAGAGAGATGGAAGGGAG	ACGTTGGATGTCGAATCACGATCTGAACAG

dbSNP rs#	Forward PCR primer	Reverse PCR primer
rs1013137	ACGTTGGATGATTACAAGCAGTGTCACTCC	ACGTTGGATGGGGTTAATGAATAGGTGGAAC
rs5878836	ACGTTGGATGTTTGGTATGGAGTGACACTG	ACGTTGGATGCCAATGATAATCTCCAGTGTC
rs1981480	ACGTTGGATGCGACTGTCTTCTTCTGCAG	ACGTTGGATGTGCTGCACCTCCCTACTCTT
rs1981479	ACGTTGGATGTGAGTAGCTAGAAGTACAGG	ACGTTGGATGATCACTGCAGCCTTAAACTC
rs3035187	ACGTTGGATGTGAGTAGCTAGAAGTACAGG	ACGTTGGATGATCACTGCAGCCTTAAACTC
rs7453993	ACGTTGGATGTGACAAAGTGAGACCAACTC	ACGTTGGATGTGGGAGATCACCTTTCATAC
rs2001119	ACGTTGGATGGCTTCTTTAGGTCTTCATTTT	ACGTTGGATGTGAGTTTGTGTTAAAGCTC
rs2001118	ACGTTGGATGGGTCCAGCCAAAAACAACC	ACGTTGGATGAGGCTGGAATTTACAAGGCC
rs2001117	ACGTTGGATGGTCCAGCCAAAAACAACCC	ACGTTGGATGAGGCTGGAATTTACAAGGCC
rs6940433	ACGTTGGATGTTGTGAGCTACCTCATTAC	ACGTTGGATGCAACATCTGGGTTATTTGTG
rs1318746	ACGTTGGATGTAAGCTGGTGCTTATTTAG	ACGTTGGATGGGTGGCCAATAAACATAAGC
rs763099	ACGTTGGATGGAGGCAAGTTGTGAAAGACC	ACGTTGGATGGGCCCTTGAAGTTTCTCAG
rs5878837	ACGTTGGATGTCACCAGCCGTATTCATCAG	ACGTTGGATGTGAAAGACCTTCTGCCCATC
rs964731	ACGTTGGATGGGAAATCATACCCCTTTCC	ACGTTGGATGTGAGGGATACTTGAGCTCTG
rs964730	ACGTTGGATGCACTCTGGCAAAGGGATTTA	ACGTTGGATGGTAGGAAAGCAGAAAGGTAC
rs6921869	ACGTTGGATGTAGTAGAGACAGGGTTTCAC	ACGTTGGATGTACTTGGGAGGCTAAGATGG
rs3945029	ACGTTGGATGCTCTTCTGTAAATCTTGCC	ACGTTGGATGAGAGAAAGGCTGAACACATG
rs4945715	ACGTTGGATGCTCAAGGGACAGTCATTGAG	ACGTTGGATGGTCAGGGTGCTCATGAATTG
rs7775252	ACGTTGGATGGACTAGGGATTGGATTTTGG	ACGTTGGATGTTTCTTCATCCAGCTATGGC
rs7742098	ACGTTGGATGGAAGAAAACAGAAAAGTGGC	ACGTTGGATGAAGAATTCGTTCTTTCCCC
rs3757289	ACGTTGGATGGCGATTTTATTTGTAGTACAG	ACGTTGGATGAATACTTGTGCCTCAAGAAG
rs6905458	ACGTTGGATGAGGAATATCAGCCTTTTGGG	ACGTTGGATGGCTCTTCTAACAGAAAGTACC
rs3757290	ACGTTGGATGTAAACATGCCAGCACAAACAG	ACGTTGGATGTGCTCCAGAGTTAATTTGTC
rs2275289	ACGTTGGATGTTGAAAAGGAACTCAGTGGC	ACGTTGGATGGTCCAGTTAGTCTTCTGAAC
rs4945716	ACGTTGGATGTAGAGCCTCACTGTGTTACC	ACGTTGGATGAATCTGGCACTTTGGGAGG
rs6922638	ACGTTGGATGGCTTAGTGTCTGTGCTTTTG	ACGTTGGATGCCTGCTGTTTCATTTTGAGG
rs7739572	ACGTTGGATGGTTTAAAGAGACATTGGGTG	ACGTTGGATGTCTATTTGGACCATGCATTC
rs6901187	ACGTTGGATGTCAGCACAGACCCTTAAATG	ACGTTGGATGGGCTTTTTTCTCACCCACC
rs4946656	ACGTTGGATGTGGCCCAGACGATATAAAGG	ACGTTGGATGATTAAGCTCCCACTTAGGC
rs1338020	ACGTTGGATGTCTGTGGTCAACAACAGTCC	ACGTTGGATGCATCTCAGGCAGGATATAGC
rs7771472	ACGTTGGATGTTACCTGAAGGTGAATCTAG	ACGTTGGATGGTACAAACCTTTTGGAAAAC
rs6926260	ACGTTGGATGTACCACAGTGCTGGGATTAC	ACGTTGGATGCGTAGAGTAGTGCATTGTGC
rs6926627	ACGTTGGATGAGGTGTGCACCCATTATCCA	ACGTTGGATGGGATACTATACCCATTTACTC
rs4946657	ACGTTGGATGCCAGGTAGAATTATTATGGG	ACGTTGGATGCCACCATTAATCACTGTATC
rs6571218	ACGTTGGATGCGCAGACACCTTATTAAAG	ACGTTGGATGTTGACAATAGGTAAGTGGC
rs7449944	ACGTTGGATGAACCTTTGTGCGCCCTGGCGG	ACGTTGGATGCCAGCGAGGAGGGACAGAG
rs952175	ACGTTGGATGATGCTCTGCCAGCCTTTTTT	ACGTTGGATGTCAAAACAGCTGGTAGGGAC
rs1890228	ACGTTGGATGTTAAGGCATTCCCATATCCT	ACGTTGGATGCCAGATGTATGAATAGTAGC
rs1933237	ACGTTGGATGGGGTTCAAGCAATTCCTGTC	ACGTTGGATGCAAAAATTAGCCCGGTGTGG
rs1338019	ACGTTGGATGTATGTGTGTACAAAAGGGAG	ACGTTGGATGCCTGCAGAATCTACAACATG
rs7453127	ACGTTGGATGCATCACCTCAGATAGTTACC	ACGTTGGATGGTGACTCCAGTTAGCTATAC
rs7381551	ACGTTGGATGAGTTTGTACCTTTGACCAC	ACGTTGGATGGTTGAACCTACAGAAACAGAG
rs6571219	ACGTTGGATGGACAGTACTGAAAGTCTTCG	ACGTTGGATGCTTCTTCTATCTGATTTGG
rs6571220	ACGTTGGATGTCTATCTGATTTGGAAGGC	ACGTTGGATGAACAAGACGAGAGTGTCTTG
rs2185017	ACGTTGGATGATGTGGGAAGATCACTTGAG	ACGTTGGATGAGCCCCGCTAATTGTATAT
rs1591720	ACGTTGGATGTTGGAATTACAGGTGTGAGC	ACGTTGGATGACAAGCCCACAGCTAACATC

dbSNP rs#	Forward PCR primer	Reverse PCR primer
rs6925046	ACGTTGGATGGCTTGCTTTTTGAGACAGGG	ACGTTGGATGTAGAGGCTGTAGTGAGCTGT
rs6940423	ACGTTGGATGGTGCTGGGATTACAGATGTG	ACGTTGGATGCCCTGTCTCAAAAAGCAAGC
rs1190274	ACGTTGGATGCATTTAGTCTCTGAGGACAAC	ACGTTGGATGCCCTTTCTAACCACTAAATACC
rs1190276	ACGTTGGATGCTGTAATCCCAGCACTTTGG	ACGTTGGATGTAGTAGAGACTGGCTTTCAC
rs1591719	ACGTTGGATGCTCACACATTCCTGAAAG	ACGTTGGATGCTGTCAGAAACTGCTCTGTC
rs1933236	ACGTTGGATGCCAAGTCATTTGAAACCTTC	ACGTTGGATGTAAGCTCAGAAAATGGCATC
rs6905202	ACGTTGGATGGTATTACAGTGTGAATCAGG	ACGTTGGATGCCCATTCACATCAATTTTC
rs1209150	ACGTTGGATGTCCTCCAGAACTTTTGACC	ACGTTGGATGGGCCTTTATTACTTGCTACC
rs1190277	ACGTTGGATGATCATGTGCTAAGCACCACG	ACGTTGGATGCCCTCCAGGTCAAAGTTTC
rs6926278	ACGTTGGATGTAGAACTCCCAGGCTCAAGA	ACGTTGGATGTATTAGCTGGGTGTAGTGGC
rs1190278	ACGTTGGATGAGATACTGAGAAGGGTAGTC	ACGTTGGATGGTGCTACTGAATACTAGATC
rs4626463	ACGTTGGATGAGAAATTGCCAACCAGCCTC	ACGTTGGATGGGTCCAGAAGCAAGACAAAG
rs6924620	ACGTTGGATGAGAACAAATGCCTGGCACATG	ACGTTGGATGTGACAGAGTGAGACTCTGTC
rs1190280	ACGTTGGATGTAGAAAGTGCCATCCAATGC	ACGTTGGATGACAACTAGGCAGACAGTAC
rs4557552	ACGTTGGATGCGTCCTTTACATAACCCAG	ACGTTGGATGCATTTTCTCGGTGACCTAGG
rs6932711	ACGTTGGATGATCACCTGCTCAAGGTCATC	ACGTTGGATGGATGGTGCAATTTGCATGCAG
rs1686140	ACGTTGGATGAGAAAGAACCCCTAGTTGGAG	ACGTTGGATGGAACATAGTCTGCATGTGATC
rs1190281	ACGTTGGATGCACCTTTTTGCTACAACCTC	ACGTTGGATGATCTCTTGCAATTTATTCTAC
rs2308162	ACGTTGGATGCACCTTTTTGCTACAACCTCC	ACGTTGGATGGCATCAAGTAACTGCACATT
rs1190282	ACGTTGGATGTATGTGGACAGTAGCAACCC	ACGTTGGATGAGACTCAGGAGTTGCTTCTC
rs1765907	ACGTTGGATGCTTCTTGAGAAGCAACTCC	ACGTTGGATGGGGAGAATGAAATTCACCTT
rs5878838	ACGTTGGATGCCCTGTCATTCAAGGCATAG	ACGTTGGATGTTGCTCAGCATCGCTACATC
rs1190283	ACGTTGGATGCCCACTGACCTACAATATAT	ACGTTGGATGGACAGATTGAAGATGGCTAG
rs1190284	ACGTTGGATGATCTTTCAAACTGCCAGAC	ACGTTGGATGGCCAGTGGATTTCAGTTGTT
rs1190285	ACGTTGGATGACTTGAGTCACAGACATAGC	ACGTTGGATGGGCTCTTGATTATTTTCTGC

TABLE 40

dbSNP rs#	Extend Primer	Term Mix
rs2400080	TCCTTTACTTTACCTTTTTTTCC	ACG
rs6930209	GATTTTTATGCAAATATCAGATGA	ACT
rs221628	AAGAATAGACATATTTGTAGATCA	ACT
rs221629	TCTCCCTCTGGCCCAACTG	ACT
rs221630	GACAGGTGATGGCTTGGGA	ACG
rs221631	TGTCAAATGGAAAGATGATTAAT	ACT
rs1149284	CTAGACACATTGTCTGCTAGT	ACT
rs221633	AACAATTTGTCTTGAGATCTTT	ACT
rs423366	ACCAAAAAACATTTTGCAGATAG	ACG
rs436460	ATATACCAAAAAACATTTTGCAGA	ACG
rs2211010	GAGACAGAGTCTCGCTCTGT	ACG
rs379908	TGGATAACACAGTGCATACCA	ACG
rs1149285	ATTTATGAAGCACAAAGAACAAC	ACT
rs7341194	ACTGGAAAAATTTTTTCCTTTGT	ACT

dbSNP rs#	Extend Primer	Term Mix
rs715153	GCCCTCTAGTGGGCTTAATG	ACT
rs221634	GCCAGGATGACCCCAAAATA	CGT
rs7757307	CCCATAATTCTTTAACTAAATAC	ACT
rs221635	ACAGTAAATGAAGGACATTGGC	ACG
rs4145418	TAGCCCTGTAAGCTGATC	CGT
rs221636		
rs3185958	CCACATCTTAAAGAGGCTGTT	ACT
rs4946654	GCCTATTGAAGAAATCATTTTAGA	CGT
rs221637	ACTTGAGCGATCCTCCAC	CGT
rs221638	CCTGGCTGAAAATCTTAAAAAAA	ACT
rs221639	GTGTGTGTGTGTGTGAACCA	ACT
rs643545	CACCCGCATGTGTATGTATCT	ACT
rs221640	TCGTCTGAAGTCAGGAGTTC	ACT
rs3957696	TCTCTCTCTCTCTCTCAC	CGT
rs3995554	TCTCTCTCTCTCTCTCACAC	CGT
rs7453502	GAGTTTGTGTTTTAAAGAACTTTT	CGT
rs1190471	AAGAGTGATAAATGACCAGGC	ACT
rs221641	GAGATGTGAGCCACTGCGC	ACT
rs221642	CCAACCATGTGGAAGTGTGA	ACT
rs1190472	TTATCAACAGCATGAAAACGGA	ACG
rs1190473	CTGCACTCCAGCCCGGGA	ACT
rs186404	GGAGACACAGTGAGACTGTC	CGT
rs221643	AATGAAGAAGTCTTGCAATTTCTT	CGT
rs221644	CTAGCTCCAAGCCAGGTTAT	ACT
rs1203475	GCAGGAGAATCGCTTGAACC	ACG
rs221645	CAGACCTCAAAGTGGTCAAGA	ACT
rs170277	GACCCTTGCTAGCACTCAGA	ACG
rs221646	CAGGCAAACAGGTCCAGAG	ACG
rs221647	AGCTCCTTTCCTTAGGTTATC	ACT
rs221648	GGCAACGGAGTGAGACCC	ACG
rs221649	AAGAAGAAGGCTGGGAGAAC	ACT
rs221650	GGCACAGTGGCTCACACTT	ACT
rs1149287	TCCCAGGCTGGTCTTGAAC	ACG
rs221651	AGCTGCAATGAGCTGTGATCG	ACG
rs7762591	CTTCCGTCTCCTGAGTTCCA	ACT
rs7748555	CAGGTGTGAGCCACCATGC	ACG
rs5878833	GCCTTCTGGCCATTTTTTTTTT	ACG
rs5878834	GCCTTCTGGCCATTTTTTTTTT	ACG
rs221652	TACTCAGGGAAGGATGTTACA	ACG
rs221653	TGTGTTAGCCAGGATGGTCT	ACG
rs221654	AAGACCATCCTGGCTAACAC	ACT
rs221655	GCTAACACGGTGAAACCCC	ACT
rs221656	CAGGCTGGAGTGTAGTGGC	ACT

dbSNP rs#	Extend Primer	Term Mix
rs221657	CACTTCCTCCCTCCGACTC	ACG
rs221658	TCAGCATTTGTGGGCTGCC	ACT
rs110065	CTCCTTGCTGGTTGTGGCA	CGT
rs221659	ATGAATTCTATCTGTGCGACC	ACG
rs221660	GGAAACCAGGGCTTTTTTTTTT	ACT
rs7742821	ATTTCCATTTGTGTTGAGTCCT	ACT
rs221662	GAAATAAAAAGGAATCACACCC	ACT
rs7748426	CACATCTGTACTATTATTTCTACT	ACT
rs6911494	AGGCCAGGCTAACTGGGG	ACG
rs6939846	GTGGCCATGACAGTTGCAG	CGT
rs368471	TTATATTTCAAGGGAATGCTCTT	ACT
rs430190	GCCTCTGGGCAAATTTCTGA	ACG
rs455114	TTTTTACAGTTGGGAGGCAGA	ACT
rs405956	AAGACTGGGACAGCAGCGA	ACT
rs5878835	GAACCACAGAAGGCCTTAAAAA	CGT
rs1473814	GAACCACAGAAGGCCTTAAAAA	CGT
rs423272	GTGGGTACATAGTGGATGTAT	ACT
rs413806	ACAGATTTACATCGTGGTACTC	ACG
rs4946655	GTAGCTTTGGCTTGTGCACC	ACT
rs6915632	CTTGACTCACTGCAACCTCA	ACT
rs2095723	TCTGTCCTCACACAGCATTTT	ACG
rs7450078	CGCTATGTTGCCAGGCTC	ACT
rs7453071	CCAAGGCAGGAGGATCTCT	ACT
rs1018810	CTGCTTTTATACATGCCACAC	ACT
rs7450944	GGGCTCCCTTTCATCTCT	ACT
rs7748657	GTAGTGGCTGAATGCGATGT	ACT
rs1013137	CACTCCATACCAAATTAAATATAC	ACG
rs5878836	GTGACACTGCTTGTAATTCTG	CGT
rs1981480	TACAATGGCAGTGACCCAGA	CGT
rs1981479	CTACAGGCCTGCACCACGA	ACG
rs3035187	ATGCCTGGCATTTTTTTTTTTTT	CGT
rs7453993	GTGAGACCAACTCCCATCC	ACG
rs2001119	CTTACAAAAGCTTCTGTGCCAT	ACT
rs2001118	CAGCCAAAAACAACCCTAAAA	ACT
rs2001117	AAAAACAACCCTAAAAAGGAAGA	ACT
rs6940433	TGCCAAGAGGCACATTTTCC	ACT
rs1318746	AGGCTACTAAGTATATTTGATTTT	ACT
rs763099	AAAGACCTTCTGCCCATCCA	CGT
rs5878837	CGTATTCATCAGCAACAGCC	ACT
rs964731	ATACCCCTTTCTTCAGTAT	ACT
rs964730	TGAGGGATACTTGAGCTCTGT	ACT
rs6921869	GTCTCGAGCTCCTGGCCT	ACT
rs3945029	ATTAGCAGCCTCCTCCACTA	ACT

dbSNP rs#	Extend Primer	Term Mix
rs4945715	CTTCTCTTTCTCCTTTTCATC	ACT
rs7775252	TTGAGAATTATTCCTCGTAATTA	ACT
rs7742098	CCAGAAACTGGCTTTGCCTT	ACT
rs3757289	AAAAATCCACAGAGATGATGG	ACT
rs6905458	CCTCTCAGAAGTGTGCCAG	ACG
rs3757290	GACTGACTCTCTCCCCAAAA	ACT
rs2275289	AGGAAGTCAAGTGGCATGTAC	ACG
rs4945716	CTCACTGTGTTACCCAGGCT	ACT
rs6922638	GTGCTTTTGTTCCTTCTCATACT	ACT
rs7739572	AGACATTGGGTGTTTCTCTTTT	ACT
rs6901187	CTGACACATAGCTGCCAGAG	ACT
rs4946656	CTGTTGAAGAGCAAAGTTAACA	ACG
rs1338020	GCAAGACATTCTGAATAGTGC	ACT
rs7771472	GAAGGTGAATCTAGGGAATGAA	CGT
rs6926260	GTAAGCCACTGTGTCCAGC	ACG
rs6926627	AAGGCAGAGCAGGGTCCC	ACT
rs4946657	GTTTCATGTTGTATCTCTCTGT	ACT
rs6571218	CCTTATTAAAGAGAGAGAGAGA	ACT
rs7449944	GGCGGCAGCTGCTTGTTT	ACT
rs952175	CTGGGCGCACTGCAACCT	ACT
rs1890228	CCATATCCTGGGCTATGTGT	ACG
rs1933237	CTTAGCCTCCAGAGTAGCTG	ACT
rs1338019	GTCACAAAGGGAGAACTCAA	ACG
rs7453127	CTACTCTCTTAGCAAATTCAGTT	ACT
rs7381551	TTCCACCCCTCAGCCCC	ACT
rs6571219	CGCTGGGGCAGAAAAAGAAA	ACG
rs6571220	TTCTTTTCTGCCCCAGCGA	ACT
rs2185017	CAACACAGTGAGCAGTGAGA	ACG
rs1591720	GTGTGAGCCACCATGCCCA	ACT
rs6925046	GACAGGGTCTTGCTCTGTC	ACT
rs6940423	GGATTACAGATGTGAGCCAC	ACG
rs1190274	GGACAACACTTTTAAAGGTACT	ACT
rs1190276	CCAGCACTTTGGGAGGCC	ACT
rs1591719	TTGAATCTCTTTTAGAGTATGG	ACT
rs1933236	ATTTCCTGATTCACACTGTAATA	ACG
rs6905202	GAAATTTTTCACGTTTGAAGGT	ACG
rs1209150	TGACCTGGAGGGAGAAAAAG	CGT
rs1190277	GCTAAGCACACGGAGATAC	ACT
rs6926278	CTCCACCTCAGCCTCCC	ACG
rs1190278	GGGTAGTCGGTAAAGGGGA	ACG
rs4626463	AGGGACTTTCCACACTAACC	ACT
rs6924620	TAAATATTCATTGCATAGAAGGAA	ACT
rs1190280	ATGCTGCATGTATTTATGGC	ACT

dbSNP rs#	Extend Primer	Term Mix
rs4557552	ACCCCAGTACTTCCTCTCC	ACG
rs6932711	GTCATCACTCCCGCAGTTCA	ACG
rs1686140	CCCTTCCTTTGGAAACTGG	ACT
rs1190281	TTTAAATGTGCAGTTACTTGATG	ACG
rs2308162	CCTCCAGTGAAAGCAATTATTT	CGT
rs1190282	GCTGAGAATACTTGCTGGCT	ACT
rs1765907	TGAGAAGCAACTCCTGAGTC	ACG
rs5878838	TGCCAATTAGCACTGAAAAAAG	ACT
rs1190283	CTACAAAATTCGTTACTACATAC	ACT
rs1190284	CAGACGTGGCAGCAGAGTAA	ACT
rs1190285	GTCACAGACATAGCCATTTAGA	ACT

Genetic Analysis

[0276] Allelotyping results from the discovery cohort are shown for cases and controls in Table 41. The allele frequency for the A2 allele is noted in the fifth and sixth columns for osteoarthritis case pools and control pools, respectively, where “AF” is allele frequency. The allele frequency for the A1 allele can be easily calculated by subtracting the A2 allele frequency from 1 (A1 AF = 1-A2 AF). For example, the SNP rs1474555 has the following case and control allele frequencies: case A1 (C) = 0.64; case A2 (T) = 0.36; control A1 (C) = 0.70; and control A2 (T) = 0.30, where the nucleotide is provided in paranthesis. Some SNPs are labeled “untyped” because of failed assays.

TABLE 41

dbSNP rs#	Position in SEQ ID NO: 4	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs2400080	241	105557091	A/G			
rs6930209	801	105557651	A/G			
rs221628	899	105557749	A/G	0.716	0.755	0.216
rs221629	2091	105558941	C/G	0.775	0.801	0.338
rs221630	2290	105559140	C/T	0.066	0.049	0.465
rs221631	2440	105559290	A/G	0.147	0.137	0.686
rs1149284	4959	105561809	G/T			
rs221633	7914	105564764	C/G	0.094	0.091	0.911
rs423366	7969	105564819	A/G	0.392	0.418	0.448
rs436460	7972	105564822	C/T	0.186	0.175	0.720
rs2211010	10831	105567681	C/T			
rs379908	12399	105569249	C/T	0.773	0.809	0.242
rs1149285	13841	105570691	C/T			
rs7341194	14461	105571311	C/T			
rs715153	14680	105571530	C/T			
rs221634	16808	105573658	A/T	0.330	0.314	0.630
rs7757307	18231	105575081	C/T			
rs221635	18394	105575244	C/T			
rs4145418	18505	105575355	G/T	0.380	0.377	0.929

dbSNP rs#	Position in SEQ ID NO: 4	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs221636	18684	105575534	A/T	0.807	0.829	0.458
rs3185958	19257	105576107	C/T			
rs4946654	20263	105577113	A/T			
rs221637	20656	105577506	A/C	0.879	0.901	0.409
rs221638	21499	105578349	A/G	0.089	0.072	0.427
rs221639	21563	105578413	A/C	0.934	0.951	0.537
rs643545	21612	105578462	C/G	0.824	0.842	0.486
rs221640	21834	105578684	C/T			
rs3957696	22406	105579256	A/T			
rs3995554	22408	105579258	A/T			
rs7453502	22685	105579535	A/T			
rs1190471	23303	105580153	C/T			
rs221641	23306	105580156	C/G	0.070	0.053	0.415
rs221642	25139	105581989	A/G	0.868	0.869	0.987
rs1190472	25211	105582061	C/T	0.227	0.191	0.244
rs1190473	25364	105582214	A/G	0.722	0.742	0.521
rs186404	25381	105582231	A/C			
rs221643	25414	105582264	A/T	0.550	0.766	~0.0001
rs221644	25835	105582685	C/T	0.695	0.774	0.007
rs1203475	26214	105583064	A/G			
rs221645	27224	105584074	A/G	0.066	0.048	0.344
rs170277	27526	105584376	A/G	0.840	0.882	0.137
rs221646	27934	105584784	C/T	0.866	0.897	0.244
rs221647	28550	105585400	C/T	0.844	0.884	0.102
rs221648	29015	105585865	A/G	0.865	0.891	0.341
rs221649	29879	105586729	G/T	0.102	0.081	0.359
rs221650	29979	105586829	A/G	0.856	0.887	0.192
rs1149287	30030	105586880	A/G			
rs221651	30585	105587435	C/T	0.177	untyped	NA
rs7762591	31753	105588603	C/G			
rs7748555	31934	105588784	C/T	0.670	0.712	0.199
rs5878833	33227	105590077	-/T	0.140	0.113	0.338
rs5878834	33228	105590078	-/T	0.142	0.114	0.309
rs221652	35172	105592022	C/T	0.172	0.120	0.064
rs221653	36901	105593751	A/G			
rs221654	36921	105593771	A/G			
rs221655	36932	105593782	A/G			
rs221656	37061	105593911	C/T			
rs221657	37570	105594420	C/T	0.924	0.953	0.218
rs221658	38745	105595595	G/T	0.043	0.028	0.421
rs110065	38970	105595820	A/T	0.834	0.894	0.031
rs221659	39725	105596575	C/T	0.048	0.027	0.347
rs221660	40070	105596920	A/C	0.841	0.878	0.133
rs7742821	40460	105597310	C/G			
rs221662	41470	105598320	A/G	0.778	0.879	~0.0001
rs7748426	41562	105598412	A/G			
rs6911494	41956	105598806	A/G	0.043	0.032	0.652
rs6939846	42047	105598897	A/T			
rs368471	42280	105599130	A/G	0.150	0.104	0.074
rs430190	42358	105599208	A/G	0.053	0.033	0.386
rs455114	42629	105599479	C/G	0.059	0.027	0.100
rs405956	43075	105599925	C/T	0.132	0.089	0.063
rs5878835	43387	105600237	-/A			
rs1473814	43393	105600243	G/T	0.126	untyped	NA
rs423272	43438	105600288	C/T	0.023	untyped	NA
rs413806	44115	105600965	A/G	0.837	0.895	0.037

dbSNP rs#	Position in SEQ ID NO: 4	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs4946655	44537	105601387	A/G	0.062	0.033	0.128
rs6915632	45642	105602492	A/G			
rs2095723	46629	105603479	A/G			
rs7450078	47496	105604346	A/G	0.261	0.163	0.001
rs7453071	47515	105604365	A/C			
rs1018810	48329	105605179	A/G			
rs7450944	48862	105605712	C/G			
rs7748657	48908	105605758	A/G	0.972	untyped	NA
rs1013137	49038	105605888	C/T	0.699	0.785	0.006
rs5878836	49080	105605930	-/T			
rs1981480	50204	105607054	A/T	0.880	0.946	0.012
rs1981479	50404	105607254	A/G	0.052	0.035	0.453
rs3035187	50426	105607276	-/TTA	0.033	untyped	NA
rs7453993	50531	105607381	C/T	0.170	0.135	0.222
rs2001119	50840	105607690	C/T	0.176	0.122	0.033
rs2001118	50964	105607814	C/T	0.793	0.883	0.001
rs2001117	50971	105607821	C/T	0.575	0.650	0.035
rs6940433	51378	105608228	C/T			
rs1318746	52610	105609460	A/C	0.140	0.089	0.171
rs763099	53906	105610756	A/T	0.865	0.922	0.029
rs5878837	53951	105610801	-/C	0.423	0.463	0.215
rs964731	54111	105610961	A/C	0.865	0.926	0.089
rs964730	54149	105610999	G/T	0.903	0.951	0.022
rs6921869	55563	105612413	C/G			
rs3945029	55999	105612849	C/T	0.972	0.976	0.820
rs4945715	58415	105615265	C/G	0.057	0.021	0.048
rs7775252	58961	105615811	C/G	0.027	untyped	NA
rs7742098	60447	105617297	C/T			
rs3757289	61377	105618227	A/G			
rs6905458	61528	105618378	A/G	0.045	0.023	0.345
rs3757290	61606	105618456	C/G			
rs2275289	62140	105618990	A/G			
rs4945716	62461	105619311	C/T			
rs6922638	63826	105620676	C/T	0.086	0.054	0.120
rs7739572	64950	105621800	G/T	0.920	0.931	0.613
rs6901187	65076	105621926	G/T	0.054	0.026	0.122
rs4946656	66121	105622971	C/T			
rs1338020	66406	105623256	C/T	0.109	0.077	0.145
rs7771472	67051	105623901	A/C	0.035	untyped	NA
rs6926260	68860	105625710	C/T	0.921	0.952	0.196
rs6926627	69014	105625864	C/T			
rs4946657	70796	105627646	C/T	0.224	0.136	0.001
rs6571218	72325	105629175	G/T	0.589	0.677	0.011
rs7449944	73414	105630264	A/C			
rs952175	75258	105632108	C/G	0.650	0.730	0.007
rs1890228	76347	105633197	A/G	0.046	0.028	0.426
rs1933237	76839	105633689	A/C	0.925	0.953	0.175
rs1338019	77358	105634208	A/G	0.888	0.930	0.101
rs7453127	77822	105634672	A/G	0.415	0.534	0.002
rs7381551	77946	105634796	G/T	0.026	untyped	NA
rs6571219	80002	105636852	A/G	0.837	0.903	0.017
rs6571220	80024	105636874	A/G	0.464	untyped	NA
rs2185017	80285	105637135	A/G	0.066	0.036	0.196
rs1591720	80397	105637247	C/G	0.027	untyped	NA
rs6925046	82075	105638925	C/T			
rs6940423	82153	105639003	A/G	0.024	0.029	0.840

dbSNP rs#	Position in SEQ ID NO: 4	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs1190274	83981	105640831	A/G	0.067	0.041	0.183
rs1190276	84184	105641034	A/G			
rs1591719	85089	105641939	C/T			
rs1933236	85288	105642138	A/G	0.892	0.942	0.046
rs6905202	85330	105642180	C/T	0.888	0.909	0.435
rs1209150	85581	105642431	A/T	0.862	0.922	0.023
rs1190277	85642	105642492	A/G	0.158	0.118	0.098
rs6926278	86433	105643283	A/G			
rs1190278	86904	105643754	A/G	0.211	0.147	0.030
rs4626463	88391	105645241	A/G	0.067	0.050	0.383
rs6924620	89042	105645892	C/T			
rs1190280	90828	105647678	G/T	0.890	0.948	0.008
rs4557552	92676	105649526	C/T	0.033	0.025	0.736
rs6932711	92881	105649731	C/T			
rs1686140	94227	105651077	G/T			
rs1190281	94585	105651435	A/G	0.914	0.950	0.140
rs2308162	94616	105651466	-/ATAA	0.127	0.072	0.035
rs1190282	94712	105651562	C/G	0.879	0.937	0.009
rs1765907	94738	105651588	A/G	0.095	0.058	0.143
rs5878838	95253	105652103	-/G			
rs1190283	95522	105652372	A/G	0.054	0.032	0.245
rs1190284	95869	105652719	G/T	0.858	0.921	0.005
rs1190285	97856	105654706	C/T	0.908	0.957	0.017
rs6931398			A/G			

[0277] Allelotyping results were considered particularly significant with a calculated p-value of less than or equal to 0.05 for allelotype results. These values are indicated in bold. The allelotyping p-values were plotted in Figure 1D for the discovery cohort. The position of each SNP on the chromosome is presented on the x-axis. The y-axis gives the negative logarithm (base 10) of the p-value comparing the estimated allele in the case group to that of the control group. The minor allele frequency of the control group for each SNP designated by an X or other symbol on the graphs in Figure 1D can be determined by consulting Table 41. For example, the left-most X on the left graph is at position 105557091. By proceeding down the Table from top to bottom and across the graphs from left to right the allele frequency associated with each symbol shown can be determined.

[0278] To aid the interpretation, multiple lines have been added to the graph. The broken horizontal lines are drawn at two common significance levels, 0.05 and 0.01. The vertical broken lines are drawn every 20kb to assist in the interpretation of distances between SNPs. Two other lines are drawn to expose linear trends in the association of SNPs to the disease. The light gray line (or generally bottom-most curve) is a nonlinear smoother through the data points on the graph using a local polynomial regression method (W.S. Cleveland, E. Grosse and W.M. Shyu (1992) Local regression models. Chapter 8 of Statistical Models in S eds J.M. Chambers and T.J. Hastie, Wadsworth & Brooks/Cole.). The black line provides a local test for excess statistical significance to identify regions of association. This was

created by use of a 10kb sliding window with 1kb step sizes. Within each window, a chi-square goodness of fit test was applied to compare the proportion of SNPs that were significant at a test wise level of 0.01, to the proportion that would be expected by chance alone (0.05 for the methods used here). Resulting p-values that were less than 10^{-8} were truncated at that value.

[0279] Finally, the exons and introns of the genes in the covered region are plotted below each graph at the appropriate chromosomal positions. The gene boundary is indicated by the broken horizontal line. The exon positions are shown as thick, unbroken bars. An arrow is placed at the 3' end of each gene to show the direction of transcription.

Example 10

In Vitro Production of Target Polypeptides

[0280] cDNA is cloned into a pIVEX 2.3-MCS vector (Roche Biochem) using a directional cloning method. A cDNA insert is prepared using PCR with forward and reverse primers having 5' restriction site tags (in frame) and 5-6 additional nucleotides in addition to 3' gene-specific portions, the latter of which is typically about twenty to about twenty-five base pairs in length. A Sal I restriction site is introduced by the forward primer and a Sma I restriction site is introduced by the reverse primer. The ends of PCR products are cut with the corresponding restriction enzymes (*i.e.*, Sal I and Sma I) and the products are gel-purified. The pIVEX 2.3-MCS vector is linearized using the same restriction enzymes, and the fragment with the correct sized fragment is isolated by gel-purification. Purified PCR product is ligated into the linearized pIVEX 2.3-MCS vector and *E. coli* cells transformed for plasmid amplification. The newly constructed expression vector is verified by restriction mapping and used for protein production.

[0281] *E. coli* lysate is reconstituted with 0.25 ml of Reconstitution Buffer, the Reaction Mix is reconstituted with 0.8 ml of Reconstitution Buffer; the Feeding Mix is reconstituted with 10.5 ml of Reconstitution Buffer; and the Energy Mix is reconstituted with 0.6 ml of Reconstitution Buffer. 0.5 ml of the Energy Mix was added to the Feeding Mix to obtain the Feeding Solution. 0.75 ml of Reaction Mix, 50 μ l of Energy Mix, and 10 μ g of the template DNA is added to the *E. coli* lysate.

[0282] Using the reaction device (Roche Biochem), 1 ml of the Reaction Solution is loaded into the reaction compartment. The reaction device is turned upside-down and 10 ml of the Feeding Solution is loaded into the feeding compartment. All lids are closed and the reaction device is loaded into the RTS500 instrument. The instrument is run at 30°C for 24 hours with a stir bar speed of 150 rpm. The pIVEX 2.3 MCS vector includes a nucleotide sequence that encodes six consecutive histidine amino acids on the C-terminal end of the target polypeptide for the purpose of protein purification. Target

polypeptide is purified by contacting the contents of reaction device with resin modified with Ni^{2+} ions. Target polypeptide is eluted from the resin with a solution containing free Ni^{2+} ions.

Example 11

Cellular Production of Target Polypeptides

[0283] Nucleic acids are cloned into DNA plasmids having phage recombination sites and target polypeptides are expressed therefrom in a variety of host cells. Alpha phage genomic DNA contains short sequences known as attP sites, and *E. coli* genomic DNA contains unique, short sequences known as attB sites. These regions share homology, allowing for integration of phage DNA into *E. coli* via directional, site-specific recombination using the phage protein Int and the *E. coli* protein IHF. Integration produces two new att sites, L and R, which flank the inserted prophage DNA. Phage excision from *E. coli* genomic DNA can also be accomplished using these two proteins with the addition of a second phage protein, Xis. DNA vectors have been produced where the integration/excision process is modified to allow for the directional integration or excision of a target DNA fragment into a backbone vector in a rapid *in vitro* reaction (Gateway™ Technology (Invitrogen, Inc.)).

[0284] A first step is to transfer the nucleic acid insert into a shuttle vector that contains attL sites surrounding the negative selection gene, ccdB (e.g. pENTER vector, Invitrogen, Inc.). This transfer process is accomplished by digesting the nucleic acid from a DNA vector used for sequencing, and to ligate it into the multicloning site of the shuttle vector, which will place it between the two attL sites while removing the negative selection gene ccdB. A second method is to amplify the nucleic acid by the polymerase chain reaction (PCR) with primers containing attB sites. The amplified fragment then is integrated into the shuttle vector using Int and IHF. A third method is to utilize a topoisomerase-mediated process, in which the nucleic acid is amplified via PCR using gene-specific primers with the 5' upstream primer containing an additional CACC sequence (e.g., TOPO® expression kit (Invitrogen, Inc.)). In conjunction with Topoisomerase I, the PCR amplified fragment can be cloned into the shuttle vector via the attL sites in the correct orientation.

[0285] Once the nucleic acid is transferred into the shuttle vector, it can be cloned into an expression vector having attR sites. Several vectors containing attR sites for expression of target polypeptide as a native polypeptide, N-fusion polypeptide, and C-fusion polypeptides are commercially available (e.g., pDEST (Invitrogen, Inc.)), and any vector can be converted into an expression vector for receiving a nucleic acid from the shuttle vector by introducing an insert having an attR site flanked by an antibiotic resistant gene for selection using the standard methods described above. Transfer of the nucleic acid from the shuttle vector is accomplished by directional recombination using Int, IHF, and Xis (LR

clonase). Then the desired sequence can be transferred to an expression vector by carrying out a one hour incubation at room temperature with Int, IHF, and Xis, a ten minute incubation at 37°C with proteinase K, transforming bacteria and allowing expression for one hour, and then plating on selective media. Generally, 90% cloning efficiency is achieved by this method. Examples of expression vectors are pDEST 14 bacterial expression vector with att7 promoter, pDEST 15 bacterial expression vector with a T7 promoter and a N-terminal GST tag, pDEST 17 bacterial vector with a T7 promoter and a N-terminal polyhistidine affinity tag, and pDEST 12.2 mammalian expression vector with a CMV promoter and neo resistance gene. These expression vectors or others like them are transformed or transfected into cells for expression of the target polypeptide or polypeptide variants. These expression vectors are often transfected, for example, into murine-transformed adipocyte cell line 3T3-L1, (ATCC), human embryonic kidney cell line 293, and rat cardiomyocyte cell line H9C2.

Nucleotide and Amino Acid Sequence Examples

[0286] Table A includes information pertaining to the incident polymorphic variant associated with osteoarthritis identified herein. Public information pertaining to the polymorphism and the genomic sequence that includes the polymorphism are indicated. The genomic sequences identified in Table A may be accessed at the http address www.ncbi.nih.gov/entrez/query.fcgi, for example, by using the publicly available SNP reference number (*e.g.*, rs910223). The chromosome position refers to the position of the SNP within NCBI's Genome Build 34, which may be accessed at the following http address: www.ncbi.nlm.nih.gov/mapview/map_search.cgi?chr=hum_chr.inf&query=. The "Contig Position" provided in Table A corresponds to a nucleotide position set forth in the contig sequence (see "Contig Accession No."), and designates the polymorphic site corresponding to the SNP reference number. The sequence containing the polymorphisms also may be referenced by the "Nucleotide Accession No." set forth in Table A. The "Sequence Identification" corresponds to cDNA sequence that encodes associated target polypeptides (*e.g.*, PADI2). The position of the SNP within the cDNA sequence is provided in the "Sequence Position" column of Table A. If the SNP falls within an exon, the corresponding amino acid position (and amino acid change, if applicable) is provided as well. Also, the allelic variation at the polymorphic site and the allelic variant identified as associated with osteoarthritis is specified in Table A. All nucleotide and polypeptide sequences referenced and accessed by the parameters set forth in Table A are incorporated herein by reference.

Table A

RS_ID	Chromosome	Chrom Position	Contig Accession No. [1]	Contig Position	Nucleotide Accession No. [2]	Sequence Position	Amino Acid Position	Locus	Locus ID	A [3]	Allelic Variability	OA Assoc. Allele
910223	1	16840936	Hs1_30840_34:10	284197	AL049569	24127		PADI2	11240	F	[A/G]	A
1367117	2	21238436	Hs2_22340_34:13	79834	NM_000384	coding-nonsynon	I98T	APOB	338	F	[A/G]	G
1024791	2	102459310	Hs2_22327_34:13	4903934	NM_003854	intron		IL1RL2	8808	R	[G/A]	G
1465621	2	175653334	Hs2_5560_34:14	25660207	NM_003387	mma-utr		WASPIP	7456	F	[T/A]	A
1018810	6	105605179	Hs6_25897_34:13	9729038	NM_007073	intron		BVES	11149	F	[A/G]	A
242392	14	54639492	Hs14_26604_34:1	36569492	NM_021255	intron		PELI2	57161	F	[C/T]	T
8818	15	71960095	Hs15_10351_34:1	45034596	NM_005576	mma-utr		LOXL1	4016	R	[G/C]	C
1395486	16	76223689	Hs16_24953_34:1	3155849	NM_033401	intron		CASPR4	85445	F	[C/T]	T
512294	X	148992251	HsX_11883_34:11	1234340	NM_004224	UTR		GPR50	9248	F	[A/G]	G

[1] Contig Accession Number which can be found in the NCBI Database:
http address: www.ncbi.nih.gov/entrez/query.fcgi

[2] Sequence Identification or Nucleotide Accession Number which can be found in the NCBI Database:
http address: www.ncbi.nih.gov/entrez/query.fcgi

[3] "A" column is the sequence orientation ("F" is forward, "R" is reverse).

[0287] Following are genomic nucleotide sequences for an *APOB* region (SEQ ID NO: 1), an *IL1RL2* region (SEQ ID NO: 2), a *WASPIP* region (SEQ ID NO: 3), a *BVES* region (SEQ ID NO: 4), a *LOXL1* region (SEQ ID NO: 5), and a *CASPR4* region (SEQ ID NO: 6). The following nucleotide representations are used throughout: "A" or "a" is adenosine, adenine, or adenylic acid; "C" or "c" is cytidine, cytosine, or cytidylic acid; "G" or "g" is guanosine, guanine, or guanylic acid; "T" or "t" is thymidine, thymine, or thymidylic acid; and "I" or "i" is inosine, hypoxanthine, or inosinic acid. Exons are indicated in italicized lower case type, introns are depicted in normal text lower case type, and polymorphic sites are depicted in bold upper case type. SNPs are designated by the following convention: "R" represents A or G, "M" represents A or C; "W" represents A or T; "Y" represents C or T; "S" represents C or G; "K" represents G or T; "V" represents A, C or G; "H" represents A, C, or T; "D" represents A, G, or T; "B" represents C, G, or T; and "N" represents A, G, C, or T.

APOB genomic sequence (SEQ ID NO: 1)

>2:21188451-21288350

```

1      attatgcaca catggtctgt aaccttttaa aatacgagtg tgggaaaaca gcacattctg
61     ccacatccct gacaaaaaat tcctgacagg tggcagccgg cctcttagca acgccaccag
121    gagcctggag ttatccaggg gccacggtgg ttcccttagg ccaggtacag ggcggagttg
181    ggagacctcc tgctgggagg aaggagccca tgaaggcagc gctcagcctc cagagccRcc
241    ctgtgacagg tcaggggaca gccttggatg ggccatgaga gcccacctcc tgtRYccctc
301    taaggtggtc ccccggtttt ccaccagact gggagactca cagggAKgca gtttgttttg
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361	tgtgctaaga	aaattttccca	agactctgtg	ctgggcaactg	agtgcagcca	catccctgca
421	caagactccc	ttctcacctg	ctcaccacagg	ccctctcaca	ctaccttggt	ccaagtggcc
481	tgatattctg	cctgctaggc	acacatagtc	tgtacccttt	taaggtacaa	gtggggaaga
541	aggacacttt	ctgtcacgtc	cattaccaca	aattcctgac	aggtggcagc	tgggctctgt
601	gggaaaagga	ccaacatgct	cagttgagct	tagcacctcc	tgaggcctcc	ttagcaaggc
661	tggagcctgg	cctgtggagg	agacagggtg	cccagctgtg	gccagaaagt	gtgcaaaggt
721	tgagggtgag	aaggtggaaa	gactatgggg	ttgggcaagg	aggtataatc	tccgcttgga
781	gcatggctgg	aggagagcag	gtaatggagg	ggtggggagg	gctcccagga	aggagggcct
841	gagcagggca	tgaacaggcc	agaaaaacag	ggtgaggaag	ggttctggga	aggaacaggc
901	caaggtgtgg	gccttggggtg	gagtttcggg	ggatgcgaag	cggaggctgt	aataatgcac
961	agcacgcctt	ctctggactg	ttttgggctc	ggacacagag	cagtccttag	gctgagagac
1021	cccaatggag	tggggacagg	caacattctt	cgtggtacct	ctgtctccag	tgggctgtt
1081	gtggggatgc	atcttctgac	aaaccgcgtc	cttttggggt	agatgagaat	tcctggagat
1141	ccaggaatgc	agccttcagg	cctggggttg	ttctccccgg	agtctctgtg	acctggcctc
1201	ccagagagct	gagggtaggt	ctgcaactggc	ccctaccttc	tgacagacac	aaagcagagc
1261	tggttgtaaa	aacttaatta	aatgaaatat	tttaacaaga	aattcctaga	acagaatgcc
1321	ctgctcgtaa	gcaagccatc	acagaaataa	aagctgacag	aaagcgtgtg	ttctgtccag
1381	gaagagaaag	tttctgcaag	aaacaagata	gtgcagagag	ctggMctgtg	ctggaccagt
1441	ggctgagcaa	gcttggttct	ggagtcaaa	ccaggcagac	tcgggacaca	gccctggggc
1501	tgtgactact	tggctcagga	atgcgtgaac	tccagaccat	aattcaggca	ttccacacac
1561	ttttcctgag	cactactgtg	gtgctgggca	gtgtgcccgg	ctctgggtat	acaatgcctt
1621	cggcgcttcac	caggtgctcc	tgtgagaggg	ctattttgca	agatgaagaa	atgggggctt
1681	agggaaagtga	tgcaacaggg	taccactgag	gcagagctgg	gactggaact	caggtctgcg
1741	aatcttcaag	tccaagctct	ttaccacctc	acctactctt	tacacaacag	ctcagttagc
1801	aaaggtgctg	aagaggaaac	tcgactgcat	ttgcaggctg	ctaaattgtg	gctggcccca
1861	tgcaaggcca	cctctggggc	tggagtgtgc	cattgtacct	tcctggggga	atgcaaccac
1921	atcctgtcct	cacactggag	tgagagtggg	cagagacact	cattcccata	ctgaggaaag
1981	ggcccgcaga	tcagagtgtg	tagcttgcat	agtggacttc	ctggaggagg	tggcacttga
2041	gaggaccttg	aatgtttctg	tagagagtca	ggggaggatg	tgcccctaca	taggagggga
2101	aagggagtaa	gccacacctg	agagtgggca	gcagagcatg	gagtgatgcg	aagtggcagg
2161	aggagcctcg	actggaggcg	agagtggagc	ctggctcagg	gactgactct	cagggaaat
2221	gttgccctct	gcatgtgtca	gtaaggatga	ggtgacagaa	acatctcttg	ctcccttgcc
2281	catctcgggt	gagtgtgaga	gaaatgcatt	gcatgcacaa	gccaaggaa	tgcccttgatt
2341	tctcctgatt	gattatattcc	caggctagaa	gggttttggg	aacagcagag	tcaaatcctt
2401	cctgccaccc	ggtgcctttg	ttccacacca	tcttccagtg	gcctcagtag	gacttggtc
2461	aggggcaacc	tgatttaaga	aaattccatc	aaaaaatcct	gacatgcaaa	gaagatcat
2521	tttaggtaaa	atgggttcaca	gggtaagagg	aaactttgtt	ttacaggaat	attctccatc
2581	agggttatctt	atattgtaaa	tttcaaatat	tctttgattt	attcaaggaa	atgccccaac
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2821	gcgggctagt	cctatgtttg	gatgactctc	tgccctctgg	attattgggg	agttgaggtt
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3541	acagaggcga	aaagcttaga	tggatatagaa	ggacccgggc	tcattcagtt	catctggagc
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3661	agtgactgtc	ccaggacccc	atggctggcg	agtggcacac	ttggaaactca	cacccagctc
3721	tgtctcccaa	gtgtgctctg	tgacctcctg	cctcttctgt	ctccgtgate	ccagaatccc
3781	tataagtcct	cctcatattg	gccctcccca	gattgaacac	catgtccagc	ctcctacatc
3841	agtgccttgt	ttattttctc	gccctgaatg	cttaggagaa	tgccctggcct	agagaagggg
3901	gttaacaaat	agctactgaa	gcaataattt	tgcttgaga	aggaagattt	catctgtcca
3961	aaaatctcag	gtgaagagga	gaagggaagca	cgaatcatt	taagtgtctc	ataaaagcca
4021	tgcaacttaa	ggctttacct	ttttctcctt	tgttttaatt	cattaaatac	ataatataac
4081	taagtcaatt	agtagtgcca	taagcaccta	caaactcacc	ctccagcatg	agaatgaggg
4141	catgagggtc	accacggtg	accatgagtt	cctcacctc	ccttccctgc	ctctccccac

4201	cagatcgtaa	cctatccgga	atgttgagtt	tagcattccc	tggcttcaaa	actataatcc
4261	tagaaaacaa	agcattttgt	tcttgatttt	aactatatta	aaagagtctg	gcagccgggc
4321	tcagtggctc	atgcctgtaa	tcccagcact	ttgggaggct	gaggtggatg	gatcatgagg
4381	tcaggagatc	aagaccatcc	tggccaatgt	ggtgaaaccc	catctctact	aaaatacaaa
4441	aaattagccg	ggcgtgggtg	cacatgccag	taatcccagc	tactctggag	gctgaggtag
4501	gggaatcgct	tgaacctggg	aggcggagg	tgcagtgagc	caagatggcg	ccactgcact
4561	ccaacctggt	aacagagcaa	gactccgtct	caaacaata	aacaaacaaa	caaaaacaaa
4621	aataaaaaa	aacacaaacc	aaaaaaccca	aaagagtctg	gcactgcatt	tcactctcca
4681	ggacttgcat	tcttattccc	tattatgttg	tggtcagatt	cctctaagta	gttgagtata
4741	attgtaattg	tacgtcattt	attttgactt	ctgtcaatag	aaaaccatcg	atgcattcat
4801	tcgctgtttg	ccaggctcca	tgtatgttaa	cctcatttaa	tctttgtaac	aactctgtaa
4861	cataagatgg	ggccactttc	tcctcctgct	Yagagttgct	taacctattt	ttctcattga
4921	tctccatttt	aatgatgaaa	actgaagggt	aggaaactca	agtgactttt	ctgaagttaa
4981	ataactcatg	aatgatgaag	cttgaatttg	agagcgggga	cttcttttga	gaggagggaa
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5101	tctctattgt	tctttccagg	atcatccaac	atcccatgtg	attatagaaa	tgttctaattg
5161	actctagctc	tgatcccact	tcaaaggctt	taggtctcct	ggagctatac	tgagctccat
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5281	atggagagag	aattgggtga	gctgggctat	tggtcagcgt	gtgctttcct	taggcactgt
5341	tctctgggta	gaatctaaac	tctggatgcc	tctgtcactt	gtgggttggt	gttcagtctg
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5461	atgaaccagt	ccaccttcaa	ggcctgagtc	atccctctca	ccccaagaat	gggctttggt
5521	ggtgtcagtc	cagggagtaa	aatggacaca	agtgtcagtg	ttagcgcta	gtgccctggc
5581	attgttgact	tgaagcactg	ctcagatctg	gggaaggcat	gagccagtct	atgtagcaga
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CASPR4 genomic sequence (SEQ ID NO: 6)

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[0288] Following are cDNA sequences for *PADI2* (SEQ ID NO: 7), *APOB* (SEQ ID NO: 8), *IL1RL2* (SEQ ID NO: 9), *WASPIP* (SEQ ID NO: 10), *BVES* (SEQ ID NO: 11 and 12), *PELI2* (SEQ ID NO: 13), *LOXL1* (SEQ ID NO: 14), *CASPR4* (SEQ ID NO: 15 and 16) and *GPR50* (SEQ ID NO: 17).

PADI2 cDNA sequence (SEQ ID NO: 7)

NM_007365 Homo sapiens peptidyl arginine deiminase, type II (PADI2), mRNA

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APOB cDNA sequence (SEQ ID NO: 8)

NM_000384 Homo sapiens apolipoprotein B (including Ag(x) antigen) (APOB), mRNA

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11221	ggagacagca	tcttcgtgtt	tcaactgcct	ttgtgtacac	caaaaacccc	aatggctatt
11281	catttctccat	ccctgtaaaa	gttttggtg	ataaattcat	tactcctggg	ctgaaactaa
11341	atgatctaaa	ttcagttctt	gtcatgccta	cgttccatgt	cccatttaca	gatcttcagg
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11461	catttgccct	caacctacca	acactccccg	aggtaaaaat	ccctgaagtt	gatgtgttaa
11521	caaaatattc	tcaaccagaa	gactccttga	ttcccttttt	tgagataacc	gtgcctgaat
11581	ctcagttaac	tgtgtccag	ttcacgcttc	caaaaagtgt	ttcagatggc	attgctgctt
11641	tggtactaaa	tgccagtagcc	aacaagatcg	cagactttga	gttgccacc	atcatcgtgc
11701	ctgagcagac	cattgagatt	ccctccatta	agttctctgt	acctgtgga	attgtcattc
11761	cttcttttca	agcactgact	gcacgcttgg	aggtagactc	tcccgtgtat	aatgccactt

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11821 ggagtgccag tttgaaaaac aaagcagatt atgttgaaac agtcctggat tccacatgca
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11941 atgggtacgtt agcctctaag actaaaggaa cacttgacac ccgtgacttc agtgcagaat
12001 atgaagaaga tggcaaatTT gaaggacttc aggaatggga aggaaaagcg cacctcaata
12061 tcaaaagccc agcgttcacc gatctccatc tgcgctacca gaaagacaag aaaggcatct
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12181 actttttctaa atggaaacttc tactacagcc ctcagtccctc tccagataaa aaactcacca
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12541 ctgggaccta ccaagagtgg aaggacaagg cccagaatct gtaccaggaa ctggtgactc
12601 aggaaggcca agccagtttc cagggactca aggataacgt gtttgatggc ttggtacgag
12661 ttactcaaaa attccatatg aaagtcaagc atctgattga ctcactcatt gattttctga
12721 actttcccaag attccagttt ccggggaaac ctgggatata cactagggag gaactttgca
12781 ctatgttcat aaggagggtg gggacggtac tgtcccaggt atattcgaaa gtccataatg
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13141 atgattatat cccatatgtt tttaaattgt tgaaagaaaa cctatgcctt aatcttcata
13201 agttcaatga atttattcaa aacgagcttc aggaagcttc tcaagagtta cagcagatcc
13261 atcaatacat tatggccctt cgtgaagaat attttgatcc aagtatagtt ggctggacag
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13681 aaagattgat tgacctgtcc attcaaaact accacacatt tctgatatac atcacggagt
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13861 gaactttcac atagcacaga aaaaaattcaa actgcctata ttgataaaac catacagtga
13921 gccagccttg cagtaggcag tagactataa gcagaagcac atatgaactg gacctgcacc
13981 aaagctggca ccagggtctg gaaggtctct gaactcagaa ggatggcatt ttttgcaagt
14041 taaagaaaat caggatctga gttattttgc taaacttggg ggaggaggaa caaataaatg
14101 gagtctttat tgtgtatcat a

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IL1RL2 cDNA sequence (SEQ ID NO: 9)

NM_003854 Homo sapiens interleukin 1 receptor-like 2 (IL1RL2), mRNA.

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1 cccgcccacg gtggcgggga aatacctagg catggaagtg gcatgacagg gctcgtgtcc
61 ctgtcatatt ttccactctc cacgaggtcc tgcgcgcttc aatcctgcag gcagcccggg
121 ttggggatgt ggtccttgct gctctgcggg ttgtccatcg ccttccact gtctgtcaca
181 gcagatggat gcaaggacat ttttatgaaa aatgagatac tttcagcaag ccagcctttt
241 gcttttaatt gtacattccc tcccataaca tctggggaag tcagtgtaac atggtataaa
301 aattctagca aaatcccagt gtccaaaatc atacagtcta gaattcacca ggacgagact
361 tggattttgt ttctcccat ggaatggggg gactcaggag tctaccaatg tgttataaag
421 ggtagagaca gctgtcatag aatacatgta aacctaaactg tttttgaaaa acattgggtg
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541 ggaaaagatg atagtctcac atgtcatctg cacttcccga agagtttgtt tttgggtcca
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901 aatctacgat gctggagagt caataacact ttggtggatg attactatga tgaatccaaa

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1081 ggagtgtcca cagcatacat tatattacag ctcccagctc cggattttcg agcttacttg
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1261 gatgggaagc tgtatgacgc ctatgtctta taccccaagc cccacaagga aagccagagg
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1561 gacgggatga aggttattct cattgagctg gagaaaatcg aggactacac agtcatgcca
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1681 gagcagtcac agtgtatgaa gaccaagttt tggaagacag tgagatacca catgccgccc
1741 agaaggtgtc ggccgtttcc tccggtccag ctgctgcagc acacaccttg ctaccgcacc
1801 gcaggcccg aactaggctc aagaagaaag aagtgtactc tcacgactgg ctaagacttg
1861 ctggactgac acctatggct ggaagatgac ttgttttgct ccatgtctcc tcattctcac
1921 acctattttc tgctgcagga tgaggctagg gttagcatcc taga

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WASPIP cDNA sequence (SEQ ID NO: 10)

NM_003387 Homo sapiens Wiskott-Aldrich syndrome protein interacting protein (WASPIP), mRNA

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1 tagaagacag caggggaact cgagaagttg gttgttttca gcagattaaa acaatacaga
61 tttatcacga agactgttga acgcataact gcccaagatg cctgtccctc cccctccagc
121 acccccgccg ccccgacgt ttgcaactgg caatacacag aagcctacct tgaataagac
181 agagcaggct gggagaaatg ctctcctttc tgatacagc aaagggaaga aactaaagaa
241 gacggtcacc aatgacagaa gtgcaccaat actggacaaa cctaaaggag ctggtgctgg
301 aggcggtggt ggtggctttg gtggaggcgg cggatttggc ggaggaggtg gtggcgagg
361 cggtggaagt tttggagggg gcggacctcc aggtctggga ggattgttcc aggcgtgaat
421 gccgaagctg agatccacgg ccaacaggga taatgattct ggaggaagcc gaccaccatt
481 gttgccaccg ggaggaagat ccacatctgc gaaacccttt tcaccccaa gtggcccagg
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601 aatgccgccc ccaaggcccg acgtgggtcc aaagcctgat agcattctcc ctccagatcc
661 tagtactcca agacccattc aatcaagtcg gcacaaccgg ggggtccccc cagtgcctcg
721 aggcgccagg cagcccagcc ccgggcccac tcctccccc tccctggaa accgcggcac
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1501 tgtaaaaacg accaaaagtt atcccagcaa actggcaaga aacgaaagcc ggagtggatc
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1621 tcttctctac ccaagctcaa gagctgttcc tgttgctatc taagaactgc ataccctcct
1681 cctgtcttct tcccttgtgc ctcatgtatg ggcaggagga aaggtgggag ggggagtggt
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2281 ttcaaaatcc agagtgtatt ttacacttac cgcacatctc cattcagact agtcacattt
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4501 atagtgtaa ggttagggta gagactaccc tagacatctg catctttgta agttagccag
4561 acaataaaga aaagcagaat gaaaaaaaaa aaaaaaaaaa aaaaa

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BVES cDNA sequence 1 (SEQ ID NO: 11)

NM_007073 Homo sapiens blood vessel epicardial substance (BVES), transcript variant A, mRNA

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1 tcaggcagcc ccagcgtccc cgggccctcg gccccaccga gtgccggctc ccgcgctctg
61 cggcggcaag ccccttggaa ttttcaaaat gaattatata gagtccagcc cattgagaga
121 atcaactgcc ataggtttta cacctgagtt agaaagtatc atacctgtgc cttccaataa
181 gaccacttgt gaaaactgga gagagataca tcactctggt tttcatgtag caaatatttg
241 ttttgcagtt gggttgggta ttccaactac tcttcacctt catatgatat ttcttagggg
301 aatgttaact ctaggatgta ccctttatat cgtctgggcc actctctacc gatgtgcctt
361 ggatataatg atctggaact ctgtgttctt ggggtgcaac attttgcac tgtcgtatct
421 tttatacaag aagagaccgg taaagattga aaaggaactc agtggcatgt accggcgatt
481 gtttgaacca ctccgtgtgc ctccagattt gttcagaaga ctaactggac agttttgcat
541 gatccaaacc ttgaaaaagg gccaaactta tgctgcagag gataaaacct cagttgatga
601 ccgtctgagt attctcttga agggaaaaat gaaggtctcc tatcgaggac attttctgca
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841 tcttattgga aaagacatca caaataagct ctactcattg aatgatccca ccttaaatga
901 taaaaaagcc aaaaagctgg aacatcagct cagcctctgc acacagatct ccatgttgga

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961 aatgaggaac agtatagcca gctccagtga cagtgcgcac ggcttgcacc agtttcttcg
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1501 tccaagttat gattcttttt cctcacagtc ttttttgttc catagcaatg aggttgtcca
1561 tttgataatt ttaacaaaca atgtaagttt aaaattgagg ctaaggtaac atgaaaaagc
1621 agggaatctc aaactttat

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BVES cDNA sequence 2 (SEQ ID NO: 12)

NM_147147 Homo sapiens blood vessel epicardial substance (BVES), transcript variant B, mRNA

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1 agagcgccga tggctgggga cccgaggtcc gcgccaccca cccgcaacct ccttccccga
61 gcctttggga acgggttgtt ggccagacaa gtcccagaaa ctgcctgctt tgaagcatga
121 ataagtgcga aaagactctt agcaatgaat ttccaaaatg aattatacag agtccagccc
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301 aaatatttgt tttgcagtgt gggttggttat tccaactact ctccaccttc atatgatatt
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1441 ttttactgag ccttctgact atgccttgtt ctcttttgag atatatattt tcacagtcaa
1501 aaaaaaaaaa aaaa

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PELI2 cDNA SEQUENCE (SEQ ID NO: 13)

NM_021255 Homo sapiens pellino homolog 2 (Drosophila) (PELI2), mRNA

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1 cagccacgac ggagcagcag cgggactggc cgccccgcgc ccccttcgcc gccgtgccct
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121 ggatcgcgcc ggaggcggcg gcgtcggcgg cggcgtcggc ggccgagcgg ggctccatgt
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241 tgggtggtct cgggtacaat ggtgctttac ccaatggaga tagaggacgg aggaaaagta
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LOXL1 cDNA sequence (SEQ ID NO: 14)

NM_005576 Homo sapiens lysyl oxidase-like 1 (LOXL1), mRNA

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CASPR4 cDNA sequence 1 (SEQ ID NO: 15)

NM_033401 Homo sapiens cell recognition protein CASPR4 (CASPR4), transcript variant 1, mRNA

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CASPR4 cDNA sequence 2 (SEQ ID NO: 16)

NM_138994 Homo sapiens cell recognition protein CASPR4 (CASPR4), transcript variant 2, mRNA

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1081 cccgtgactt ttctgagctc caggagttaa ttagcactgc cagacttctc tggagaggag
1141 gaggtttctg ccacttttca atttcgaact tggaaataag cagggttctt gctgttcagt
1201 gaacttcagc tgatttcagg gggatccctc ctctttctga gtgatggaaa acttaagtcg
1261 aatctctacc agccaggaaa attaccagat gacatcacag caggtgtcga attaaatgat
1321 gggcagtggc attctgtctc tttatctgct aaaaagaatc acttgagtgt ggcggtggac
1381 ggccagatgg cttctgtctc tctctgtctg gggcctgagc agatttatte ggggtggacc
1441 tattattttg gaggttgctc tgacaaaagc tttggatcca aatgtaaaag tccacttggt
1501 ggatttcagg gatgtatgag gctcatttct atcagcggca aagtggtaga tctgatttca
1561 gtccagcagg ggtcccttgg gaacttcagt gaccttcaga tagactcatg tggcatctca
1621 gacaggtgtt tgcccaacta ttgtgaacac ggtggggagt gttcccagtc ctggagcacc
1681 tttcattgta actgtaccaa cactggttac agaggagcta cttgccataa ctctatctat
1741 gagcagtcac gtgaagccta taagcacaga ggaataactt cagggtttta ctatatgat
1801 tcagatggaa gtgggtccctt ggaaccattt cttctatatt gcaatatgac cgaaactgca
1861 tggaccatca tacagcacia cggctctgac ttaacaagag tcagaaatac taatccagag
1921 aaccatcatg ctgggttttt cgagtatgtg gccagcatgg agcaacttca ggccactatt
1981 aaccgtgcag agcactgtga acaggagtgt acttattact gcaagaagtc acggctggtc
2041 aataagcaag atggaacccc tctgagttgg tgggtaggaa gaaccaatga aacgcaaac
2101 tactggggag gttcttcgcc tgatcttcaa aaatgtactt gtggattaga gggaaactgc
2161 attgattctc agtattactg caattgtgat gctgaccgga atgaatgggt atttccatac
2221 gatttcttta tgcaagaaaa agttcattta aaaaaattaa tcactcaaag tatgtatagc

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2281 tagccagata ctgaacaagt tagtgcaatg aagtaattaa ataaagggtt gttttaatg

GPR50 cDNA sequence (SEQ ID NO: 17)

NM_004224 Homo sapiens G protein-coupled receptor 50 (GPR50), mRNA

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1  tgtttgctgt ctggacctgg ctgctgatcc tgagcctgct gggagatcct aacgatcccc
61  aggagcaaca tggggcccac cctagcggtt cccaccccct atggctgtat tggctgtaag
121 ctacccagc cagaataccc accggctcta atcatcttta tgttctgctg gatggttata
181 accatcggtg tagacctaat cggcaactcc atggtcattt tggctgtgac gaagaacaag
241 aagctccgga attctggcaa catcttcgtg gtcagtctct ctgtggccga tatgctggtg
301 gccatctacc catacccttt gatgctgcat gccatgtcca ttgggggctg ggatctgagc
361 cagttacagt gccagatggt cgggttcata acagggctga gtgtggtcgg ctccatcttc
421 aacatcggtg caatcgctat caaccgttac tgctacatct gccacagcct ccagtagcaa
481 cggatcttca gtgtgcgcaa tacctgcata tacctggtca tcacctggat catgaccgtc
541 ctggctgttc tgcccaacat gtacattggc accatcgagt acgatcctcg cacctacacc
601 tgcattctta actatctgaa caaccctgtc ttactgttta ccactgctcg catccacttc
661 gtccctccctc tcctcatcgt gggtttctgc tacgtgagga tctggaccaa agtgctggcg
721 gccctgaccc ctgcagggca gaatcctgac aaccaacttg ctgagggttc caattttcta
781 accatggttg tgatcttctc cctctttgca gtgtgctggt gccctatcaa cgtgctcact
841 gtcttggttg ctgtcagtcg gaaggagatg gcaggcaaga tccccaaact gctttatctt
901 gcagcctact tcatagccta cttcaacagc tgcctcaacg ctgtgatcta cgggctcctc
961 aatgagaatt tccgaagaga atactggacc atcttccatg ctatgcggca cccatcata
1021 ttcttccctg gcctcatcag tgatattcgt gagatgcagg aggcccgtag cctggcccg
1081 gccctgccc atgctcgcga ccaagctcgt gaacaagacc gtgcccatgc ctgtcctgct
1141 gtggaggaaa ccccgatgaa tgtccggaat gttccattac ctggtgatgc tgcagctggc
1201 caccocgacc gtgcctcttg ccaccctaag cccatttcca gatcctctc tgcctatcgc
1261 aaatctgcct ctaccacca caagtctgtc tttagccact ccaaggctgc ctctggtcac
1321 ctcaagcctg tctctggcca ctccaagcct gcctctggtc accccaagtc tgccactgtc
1381 taccctaagc ctgcctctgt ccatttcaag ggtgactctg tccatttcaa gggtgactct
1441 gtccatttca agcctgactc tgttcatttc aagcctgctt ccagcaaccc caagccatc
1501 actggccacc atgtctctgc tggcagccac tccaagtctg ccttcagtgc tgccaccagc
1561 caccctaaac ccatcaagcc agctaccagc catgctgagc ccaccactgc tgactatccc
1621 aagcctgcca ctaccagcca ccctaagccc gctgctgctg acaaccctga gctctctgcc
1681 tccatttgcc ccgagatccc tgccattgcc caccctgtgt ctgacgacag tgacctccct
1741 gagtcggcct ctagccctgc cgctgggccc accaagcctg ctgccagcca gctggagtct
1801 gacaccatcg ctgaccttcc tgacctact gtagtcacta ccagtaccaa tgattaccat
1861 gatgtcgtgg ttgttgatgt tgaagatgat cctgatgaaa tggctgtgtg aaaaatgctc
1921 tcgtaggtgg ccaggcagt

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[0289] Following are amino acid sequences for *PADI2* (SEQ ID NO: 18), *APOB* (SEQ ID NO: 19), *IL1RL2* (SEQ ID NO: 20), *WASPIP* (SEQ ID NO: 21), *BVES* (SEQ ID NO: 22), *PELI2* (SEQ ID NO: 23), *LOXLI* (SEQ ID NO: 24), *CASPR4* (SEQ ID NO: 25 and 26) and *GPR50* (SEQ ID NO: 27).

PADI2 amino acid sequence (SEQ ID NO: 18)

NP_031391 peptidyl arginine deiminase, type II; protein arginine deiminase [Homo sapiens]

MLRERTVRLQYGSRVEAVYVLGTYLWTDVYSAAPAGAQTFSCLKHSEHVWVEVVRDG
EAEVATNGKQRWLLSPSTTLRVMTMSQASTEASSDKVTVNYYDEEGSIPIDQAGLFLTAI
EISLDVDADRDGVVEKNNPKKASWTWGPEGQGAILLVNCBRETPWLPKEDCRDEKVY
SKEDLDKMSQMILRTKGPDRLPAGYEIVLYISMSDSKVGVFYVENPFFGQRYIHILGRR

KLYHVVKYTGGSSELLFFVEGLCFPDEGFSGLVSIHVSLLLEYMAQDIPLTPIFTDTVIFRI
APWIMTPNILPPVSFVCCMKDNYLFLKEVKNLVEKTNCELKVCFQYLNRGDRWIQDE
IEFGYIEAPHKGFPVVLDSPRDGNLKDFPVKELLGPDFGYVTREPLFESVTSLSDFGNLE
VSPPVTVNGKTYPLGRILIGSSFPLSGGRRMTKVVRDFLKAQQVQAPVELYSDWLTVGH
VDEFMSFVPIPGTKKFLLLMASTSACYKLFREKQKDGHGAEIMFKGLGGMSSKRITINKI
LSNESLVQENLYFQRCLDWNRDILKKELGLTEQDIIDLPAFLKMDDEDHRARAFFPNMVN
MIVLDKDLGIPKPGPQVEEECCLEMHVRGLLEPLGLECTFIDDISAYHKFLGEVHCGTN
VRRKPFTFKWLHMVP

APOB amino acid sequence (SEQ ID NO: 19)

NP_000375 apolipoprotein B precursor; apoB-100; apoB-48 [Homo sapiens]

MDPPRPALLALLALPALLLLLLAGARAEEMLENVSLVCPKDATRFKHLRKYTYNYEA
ESSSGVPGTADSRSATRINCKVELEVQLCSFILKTSQCTLKEVYGFNPEGKALLKKTKN
SEEFAAAMSRYELKLAIEPGKQVFLYPEKDEPTYILNIKRGIISALLVPPETEEAKQVLFL
DTVYGNCSHTFTVKTRKGNVATEISTERDLGQCDRFKPIRTGISPLALIKGMTRPLSTLIS
SSQSCQYTLDARKKHVAEAEICKEQHFLPFSYNNKYGMVAQVTQTLKLEDTPKINSRFF
GEGTKKMGLAFESTKSTSPPKQAEAVLKTQLQELKKLTISEQNIQRANLFNKLVTLELRGLS
DEAVTSLLPQLIEVSSPITLQALVQCGQPQCSTHILQWLKRVHANPLIDVVTYLVALIE
PSAQQRLREIFNMARDQRSRATLYALSHAVNNYHKTNPTGTQELLDIANYLMEQIQDDC
TGDEDYTYLILRVIGNMGQTMEQLTPELKSSILKCVQSTKPSLMIQKAAIQALRKMEPK
DKDQEVLLQTFLDDASPGDKRLAAYLMLMRSPSQADINKIVQILPWEQNEQVKNFVAS
HIANILNSEELDIQDLKKLVKEALKESQLPTVMDFRKFSRNYQLYKSVSLPSLDPASAKI
EGNLIFDPNNYLPKESMLKTTTLTAFGFASADLIEIGLEGKGFEPTLEALFGKQGFFPDSVN
KALYWVNGQVPDGVSKVLVDHFGYTKDDKHEQDMVNGIMLSVEKLIKDLKSKEVPE
ARAYLRILGEELGFASLHDLQLLGKLLLMGARTLQGIPQMIGEVIRKGSKNDFFLHYIFM
ENAFELPTGAGLQLQISSSGVIAPGAKAGVKLEVANMQAELVAKPSVSVEFVTNMGIIP
DFARSGVQMNTNFFHESGLEAHVALKAGKLFIPSPKRPVKLLSGGNTLHLVSTTKTE
VIPPLIENRQSWSVCKQVFPGLNYCTSGAYSNASSTDSASYPLTGDTRLELELRPTGEIE
QYSVSATYELQREDRALVDTLKFVTQAEGAKQTEATMTFKYNRQSMTLSSSEVQIPDFD
VDLGTILRVNDESTEGKTSYRLTLDIQNKKITEVALMGHLSCDTKEERKIKGVISIPRLQA
EARSEILAHWSPAKLLLQMDSSATAYGSTVSKRVAWHYDEEKIEFEWNTGTNVDTKK
MTSNFPVDLSDYPKSLHMYANRLDHRVPETDMTFRHVGSKLIVAMSSWLQKASGSLP
YTQTLQDHLNSLKEFNLQNMGLPDFHIPENLFLKSDGRVKYTLNKNLSLKIEIPLPFGGKS
SRDLKMLETVRTPALHFKSVGFHLPSREFQVPTFTIPKLYQLQVPLLGVLDLSTNVYSNL
YNWSASYSGGNTSTDHFSRLRARYHMKADSVDLLSYNVQGSGETTYDHNKNTFTLSCD
GSLRHKFLDSNIKFHVEKLGNNPVSKGLLIFDASSSWGPQMSASVHLDSSKKKQHLFVK
EVKIDGQFRVSSFYAKGTYGLSCQRDPNTGRLNGESNLRFNSSYLQGTNQITGRYEDGT
LSLTSTSDLQSGIHKNTASLKYENYELTLKSDTNGKYKNFATSNKMDMTFSKQNALLRS
EYQADYESLRFFSLLSGSLNSHGLELNADILGTDKINSGAHKATLRIGQDGISTSATTNL
KCSLLVLENELNAELGLSGASMKLTTNGRFREHNAKFSLDGKAALTELSLGSAYQAMI
LGVDKKNIFNFKVSQEGKLKLSNDMMGSYAEMKFDHTNSLNIAGLSLDFSSKLDNIYSSD
KFYKQTVNLQLQPYSLVTTLNSDLKYNALDLTNNGKLRLEPLKLHVAGNLKGAYQNN
EIKHIYAISSAALSASYKADTVAKVQGVFESHRLNTDIAGLASAIDMSTNYNSDSLHFSN

VFRSVMAPFTMTIDAHTNGNGKLALWGEHTGQLYSKFLKAEPLAFTFSHDYKGSTSH
HLVSRKSISAALEHKVSALLTPAEQTGTWKLKTQFNNNEYSQDLDAYNTKDKIGVELT
GRTLADLTLLDSPIKVPLLLSEPINIIDALEMRDAVEKPQEFTIVAFVKYDKNQDVHSINL
PFFETLQEYFERNRQTIIVVENVQRNLKHINIDQFVRKYRAALGKLPQQANDYLNFSN
WERQVSHAKEKLTALTCKYRITENDIQIALDDAKINFNEKLSQLQTYMIQFDQYIKDSY
DLHDLKIAIANIIDEIIEKLSLDEHYHIRVNLVKTIHDLHLFIENIDFNKSGSSTASWIQNV
DTKYQIRIQIEKQLQQLKRHIQNIDIQHLAGKLKQHIEAIDVRVLLDQLGTTISFERINDVL
EHVKHFVINLIGDFEVAEKINAFRAKVHELIEREYVDQQIQVLMDKLVELTHQYKLKETI
QKLSNVLQQVKIKDYFEKLVGFIDDAVKKLNELSFKTFIEDVNKFLDMLIKKLKSFYH
QFVDETNDKIREVTQRLNGEIQALELPQKAEALKLFLEETKATVAVYLESLODTKITLIIN
WLQEALSSASLAHMKAKFRETLEDTRDRMYQMDIQQELQRYLSLVGQVYSTLVTYISD
WWTLAAKNLTDFAEQYSIQDWAKRMKALVEQGFTVPEIKTILGTMPAFEVSLQALQK
ATFQTPDFIVPLTDLRIPSVQINFKDLKNIKIPSRFSTPEFTILNTFHIPSFTIDFVEMKVKIIR
TIDQMQNSSELQWPVPDIYLRDLKVEDIPLARITLPDFRLPEIAIPEFIPTLNLNDFQVPDL
HIPEFQLPHISHTIEVPTFGKLYSILKIQSPLFTLDANADIGNGTTSANEAGIAASITAKGES
KLEVLNFDQANAQLSNPKINPLALKESVKFSSKYLRTHEGSEMLFFGNAIEGKSNTVAS
LHTEKNTLELSNGVIVKINNQLTLDSENTKYFHKLNIPKLDSSQADLRNEIKTLLKAGHI
AWTSSGKGSWKWACPRFSDEGTHESQISFTIEGPLTSFGLSNKINSKHLRVNQNLVYES
GSLNFSKLEIQSQVDSQHVGHSVLTAKGMALFGEKAEFTGRHDAHLNGKVIGTLKNS
LFFSAQPFEITASTNNEGNLKVRFPLRLTGKIDFLNNYALFLSPSAQQASWQVSARFNQY
KYNQNFSAAGNNENIMEAHVGINGEANLDFLNIPLTIPEMRLPYTIITTPPLKDFSLWEKTG
LKEFLKTTKQSFDSLVSQAQYKKNKHRHSITNPLAVLCEFISQSIKSFDRHFEKNRNNALD
FVTKSYNETKIKFDKYKAEKSHDELPRTFQIPGYTVPVNVNEVSPFTIEMSAFGYVFPKA
VSMPSFSILGSDVRVPSYTLILPSLELPVLHVPRNLKLSLPHFKELCTISHIFIPAMGNITYD
FSFKSSVITLNTNAELFNQSDIVAHLLSSSSSVIDALQYKLEGTTRLTRKRGLKLATALSL
SNKFVEGSHNSTVSLTTKNMEVSVAKTTKAEIPILRMNFKQELNGNTKSKPTVSSSMEF
KYDFNSSMLYSTAKGAVDHKLSLESLSYFSIESSTKGDVKGSVLSREYSGTIASEANTY
LNSKSTRSSVKLQGTSKIDDIWNLEVKENFAGEATLQRIYSLWEHSTKNHLQLEGLFFT
NGEHTSKATLELSPWQMSALVQVHASQPSSFHDFPDLGQEVNANTKNQKIRWKNE
VRIHSGSFQSQVELSNDQEKALHLDIAGSLEGLHRLFLKNILPVYDKSLWDFLKLDDVTTSI
GRRQHLRVSTAFVYTKNPNGYSFSIPVKVLADKFITPGLKLNLDLNSVLVMPFTHVPFTD
LQVPSCKLDFREIQIYKKLRTSSFALNLPTLPEVKFPEVDVLTKEYSQPEDSLIPFEITVPES
QLTVSQFTLPKSVSDGIAALDLNAVANKIADFELPTIIVPEQTIEIPSIKFSVPAGIVIPSFQA
LTARFEVDSPVYNATWSASLKNKADYVETVLDSTCSSTVQFLEYELNVLGTHKIEDGTL
ASKTKGTLAHRDFSAEYEEDGKFEGLEWEGKAHLNIKSPAFTDLHLRYQKDKKGISTS
AASPAVGTVGMDMDDEDDDFSKWNFYSPQSSPDKKLTIFKTEL RVRESDEETQIKVNW
EEEEASGLLTSKDNVPKATGVLYDYVNKYHWEHTGLTLREVSSKLRRNLQNNAEWV
YQGAIRQIDDDVRFQKAASGTTGTQYQEWKDKAQNLYQELLTQEGQASFGQLKDNVFD
GLVRVTQKFHMKVKHLIDSLIDFLNFRFQFPGKPGIYTREELCTMFIREVGTVLSQVYS
KVHNGSEILFSYFQDLVITLPFELRKHKLIDVISMYRELLKDLSKEAQEVFKAIQSLKTTE
VLRNLQDLLQFIFQLIEDNIKQLKEMKFTYLINYIQDEINTIFNDYIPYVFKLLKENLCLNL
HKFNEFIQNELQEASQELQQIHQYIMALREEYFDPSIVGWTVKYEELEEKIVSLIKNLLV
ALKDFHSEYIVSASNFTSQLSSQVEQFLHRNIQEYLSILTPDGKGKEKIAELSATAQEIIK
SQAIATKKIISDYHQFRYKLQDFSDQLSDYYEKFIAESKRLIDLSIQNYHTFLIYITELLK
KLQSTTVMNPMYMKLAPGELTIL

IL1RL2 amino acid sequence (SEQ ID NO: 20)

NP_003845 interleukin 1 receptor-like 2 precursor; interleukin-1 receptor-related protein 2 [Homo sapiens]

MWSLLLCGLSIALPLSVTADGCKDIFMKNEILSASQPFAFNCTFPPITSGEVSVTWYKNS
SKIPVSKIIQSRIHQDETWILFLPMEWGD SGVYQCVIKGRDSCHRIHVNLTVFEKHWCDT
SIGGLPNLSDEYKQILHLGKDDSLTCHLHFPKSCVLGPIKWKDCNEIKGERFTVLETRL
LVS NVSAEDRGNYACQAILTHSGKQYEVLNGITVSITERAGYGGSVPKIIPKNHSIEVQ
LGTTLIVDCNVTDTKDNTNLR CWRVNTLVDDY YDESKRIREGVETHVSFREHNLYTV
NITFLEV KMEDYGLPFMCHAGVSTAYIILQLPAPDFRAYLIGGLIALVAVAVSVVYIYNI
FKIDIVLWYRSAFHSTETIVDGKLYDAYVLYPKPHKESQRHAVDALVLNILEVLERQC
GYKLFIFGRDEFPGQAVANVIDENVKLCRRLIVIVVPESLGFGLLKNLSEEQIAVYSALIQ
DGMKVILIELEKIEDYTVMPESIQYIKQKHGAIRWHGDFTEQSQCMKTKFWKTVRYHM
PPRRCRPFPPVQLLQHTPCYRTAGPELGSRKKCTLTTG

WASPIP amino acid sequence (SEQ ID NO: 21)

NP_003378 WASP-interacting protein [Homo sapiens]

MPVPPPPAPPPPTFALANTEKPTLNKTEQAGRNALLSDISKGKKLKKTVTNDRSAPILD
KPKGAGAGGGGGGGFGGGGGFGGGGGGGGGGSGFGGGGPPGLGGLFQAGMPKLRSTAN
RDNDSSGSRPPLLPPGGRSTSAKPFSPSPGPRFPVPSPGHRSGPPEPQRNRMPPPRPDVG
SKPDSIPPPVPSTPRPIQSSPHNRGSPVPVGGPRQPSPGPTPPPFPGNRGTALGGGSIRQSPL
SSSSPFSNRPLPPTPSRALDDKPPPPPPVGNRPSIHREAVPPPPPPQNNKPPVPSTPRPSAS
SQAPPPPPPSRPGPPPLPPSSGNDET PRLPQRNLSLSSSTPPLPSPGRSGPLPPPPSERPPP
PVRDPPGRSGPLPPPPVSRNGSTSRALPATPQLPSRSGVDSRSGPRPPLPPDRPSAGAPP
PPPPSTSIRNGFQDSPCEDEWESRFYFHPISDLPPPEPYVQTTKSYPSKLARNESRSGSNRR
ERGAPPLPIPR

BVES amino acid sequence (SEQ ID NO: 22)

NP_009004 blood vessel epicardial substance; popeye protein 1; popeye domain containing 1 [Homo sapiens]

MNYTESSPLRESTAIGFTPELESIIIPVPSNKTTCENWREIHHLVFHVANICFAVGLVIPTTL
HLHMIFLRGMLTLGCTLYIVWATLYRCALDIMIWN SVFLGVNHLHLSYLLYKKRPVKIE
KELSGMYRRLFEPLRVPPDLFRRLTGQFCMIQTLKKGQTYAAEDKTSVDDRLSILLKGK
MKVSYRGHFLHNIYPCAFIDSPEFRSTQMHKGEKFQVTIIADDNCRFLCWSRERLTYFLE
SEPFLYEIFRYLIGKDITNKLYSLNDPTLNDKKAKKLEHQLSLCTQISMLEMNRNSIASSSD
SDDGLHQFLRGTTSSMSSLHVSSPHQRASAKMKPIEEGAEDDDDDVFEPASPNTLKVHQLP

PELI2 amino acid sequence (SEQ ID NO: 23)

NP_067078 pellino 2 [Homo sapiens]

MFSPGQEEHCAPNKEPVKYGELVVLGYNGALPNGDRGRRKSRFALYKRPKANGVKPS
TVHVISTPQASKAISCKGQHSISYTLNRNQTVVVEYTHDKDMDMFQVGRSTESPIDFVVT
DTISGSQNTDEAQITQSTISRFACRIVCDRNEPYTARIFAAGFDSSKNIFLGEKAAKWKNP
DGHMDGLTTNGVLVMHPRGGFTEESQPGVWREISVCGDVYTLRETRSAQQRGKLVES
ETNVLQDGSIDLCLGATLLWRTADGLFHTPTQKHIEALRQEINAARPQCPVGLNTLAFPS
INRKEVVEEKQPWAYLSCGHVHGYHNWGHRSDEANERECPMCRTVGPYVPLWLGC
EAGFYVDAGPPTHAFTPCGHVCSEKSAKYWSQIPLPHGTHAFHAACPFCAATQLVGEQN
CIKLIFQGPID

LOXL1 amino acid sequence (SEQ ID NO: 24)

NP_005567 lysyl oxidase-like 1 [Homo sapiens]

MALARGSRQLGALVWGACLCVLVHGQQAQPGQGSDDPARWRQLIQWENNGQVYSLLN
SGSEYVPAGPQRSESSSRVLLAGAPQAQQRSHGSPRRRQAPSLPLPGRVGSDDTVRGQA
RHPFGFGQVPDNWREVAVGDSTGMALARTSVSQQRHGGSSASSVSASAFASYRQQPSY
PQQFPYPQAPFVSQYENYDPASRTYDQGFVYYRPAGGGVGAGAAVASAGVIYPYQPR
ARYEEYGGGEELPEYPPQGFYPAPERPYVPPPPPPDGLDRRYSHSLYSEGTPGFEQAYP
DPGPEAAQAHGGDPRLGWYPPYANPPPEAYGPPRALEPPYLPVRSSDTPPPGGERNGAQ
QGRLSVGSVYRPNQNGRGLPDLVPDPNYVQASTYVQRAHLYSLRCAAEEKCLASTAY
APEATDYDVRVLLRFPQRVKNQGTADFLPNRPRHTWEWHSCHQHYHSMDEFSHYDLL
DAATGKKVAEGHKASFCELDSTCDFGNLKRYACTSHTQGLSPGCYDTYNADIDCQWID
ITDVQPGRNYILKVHVNPKYIVLESDFTNVVRNCNIHYTGRYVVSATNCKIVQS

CASPR4 amino acid sequence 1 (SEQ ID NO: 25)

NP_207837 cell recognition protein CASPR4 isoform 1; contactin associated protein-like 4 [Homo sapiens].

MLLFYLLVVLSDSTKASALTNPNVALFLLADDCDDPLVSALPQASFSSSELSSSHGPGF
ARLNRRDGAGGWSPLVSNKYQWLQIDLGERMEVTAVATQGGYGSSNWVTSYLLMFS
DSGWNWKQYRQEDSIWGFSGNANADSVVYYRLQPSIKARFLRFIPLEWNPKGRIQMRI
EVFGCAYRSEVVDLDGKSSLLYRFDQKSLSPIKDIIISLKFKTMQSDGILLHREGPNGDHIT
LQLRRARLFLLINSGEAKLPSTSTLVNLTLSGLLDDQHWHSVLIQRLGKQVNFTVDEHR
HHFHARGEFNLMNLDYEISFGGIPAPGKSVSFPHRNFHGCLENLYYNGVDIIDLAKQQK
PQIIAMGNVSFSCSQPQSMPTLSSRSYLALPDFSGEEVSATFQFRTWNKAGLLLFSEL
QLISGGILLFLSDGKLKSNLYQPGKLPSDITAGVELNDGQWHSVLSAKKNHLSVAVDG
QMASAAPLLGPEQIYSGGTYYFGGCPDKSFGSKCKSPLGGFQGCMLRISISGKVVDLISV
QQGSLGNFSDLQIDSCGISDRCLPNYCEHGGECSSQSWSTFHCNCTNTGYRGATCHNSIY
EQSCEAYKHRGNTSGFYIDSDGSGPLEPFLLYCNMTETAWTIIQHNGSDLTRVRNTNP

ENPYAGFFEYVASMEQLQATINRAEHCEQEFTYYCKKSRLV NKQDGTPLSWWVGRTN
ETQTYWGGSSPDLQKCTCGLEGNCIDSQYYCNC DADRNEW TNDTGLLAYKEHLPVTKI
VITDTGRLHSEAAAYKLGPLLRCGDRSFWNSASFDTEASYLHFPTFHGELSADV SFFFKTT
ASSGVFLENLGIADFIRIELRSPTVVTFSDVGN GPFEISVQSPTHFNDNQWHHVRVERN
MKEASLQVDQLTPKTQPAPADGHVLLQLNSQLFVGGTATRQRGFLGCIRSLQLNGMTL
DLEERAQVTPEVQPGCRGHCSSYGKLCRN GGKCRERPIGFFCDCTFSAYTGPFC SNEISA
YFGSGSSVIYNFQENYLLSKNSSSHAASFH GDMKLSREMIKFSFRTRTPSLLL FVSSFYK
EYLSVIIAKNGSLQIRYKLNKYQEPDVVN FDFKNMADGQLHHIMINREEGVVFIEIDNR
RRQVHLSSGTEFS AVKSLVLGRILEHSDVDQETALAGA QGFTGCLSAVQLSHVAPL KAA
LHP SHPDPVTVTGHVTESSCMAQPGTDATSRERTHSFADHSGTIDDR EPLANAIKSDSA
VIGGLIAVVIFILLCITAIAVRIYQQKRLYKRSEAKRSENVDSAEAVLKSELNIQNAV NEN
QKEYFF

CASPR4 amino acid sequence 2 (SEQ ID NO: 26)

NP_620481 cell recognition protein CASPR4 isoform 2; contactin associated protein-like 4 [Homo sapiens]

MWNYDCDDPLVSALPQASFSSSELSSSHGPGFARLNRRDGAGGWSPLVSNKYQWLQI
DLGERMEVTAVATQGGYGSSNWVTSYLLMFSDSGWNWKQYRQEDSIWGFSGNANAD
SVVYYRLQPSIKARFLRFIPLEWNP KGRIGMRIEVFGCAYRSEVVDLDGKSSLLYRFDQK
SLSPIKDII SLKFKTMQSDGILLHREGPNGDHITLQLRRARLFL LINSGEAKLPSTSTLVNL
TLGSLDDQHWHSVLIQRLGKQVNFTVDEHRHHFHARGEFNLMNLDYEISFGGIPAPG
KSVSFPHRNFHGCLENLYYNGVDIIDLAKQQKPQIIAMGNVSFSCSQPQSMPVTFLSSRS
YLALPDFSGEEV SATFQFR TWNKAGLLLFSELQLISGGILLFLSDGKLKSNLYQPGKLP
SDITAGVELNDGQWHSVLSAKKNHLSVAVDGQMASAAPLLGPEQIYSGGTY YFGGCP
DKSFGSKCKSPLGGFQGCMRLISISGKVVDLISVQQGSLGNFSDLQIDSCGISDRCLPNYC
EHGGECSQSWSTFHCNCTNTGYRGATCHNSIYEQSC EAYKHRGNTSGFYIDSDGSGPL
EPFLLYCNMTETA WTHIQHNGSDLTRVRNTNPENPYAGFFEYVASMEQLQATINRAEH
CEQEFTYYCKKSRLV NKQDGTPLSWWVGRTNETQTYWGGSSPDLQKCTCGLEGNCIDS
QYYCNC DADRNEW

GPR50 amino acid sequence (SEQ ID NO: 27)

NP_004215 G protein-coupled receptor 50 [Homo sapiens].

MGPTLAVPTPYGCIGCKLPQPEYPPALIIFMFCAMVITIVVDLIGNSMVILAVTKNKKLR
NSGNIFVVSLSVADMLVAIYPYPLMLHAMSIGGWDLSQLQCQMVGFITGLSVVGSIFNI
VAIAINRYCYICHSLQYERIFSVRNTCIYLVITWIMTVLAVLPNMYIGTIEYDPRTYTCIFN
YLNNPVFTVTIVCIHFVLP LLIVGFCYVRIWTKVLAARDPAGQNP DNQLAEVRNFLTMF
VIFLLFAVCWCPINVLTVLVAVSPKEMAGKIPNWLYLAAYFIA YFNSCLNAVITYG LLNE
NFRREYWTIFHAMRHPIIFFPGLISDI REMQEARTLARARAHARDQAREQDRAHACPAV
EETPMNVRNVPLPGDAAAGHPDRASGHPKPHSRSSSAYRKSASTHHKSVF SHSKAASG
HLKPVSGHSKPASGHPKSATVYPKPASVHFKGDSVHFKGDSVHFKPDSVHFKPASSNP K

PITGHHVSAGSHSKSAFSAATSHPKPIKPATSHAEPPTADYPKPATTSHPKPAAADNP
SASHCPEIPAIAHPVSDDSDLPESASSPAAGPTKPAASQLESDTIADLPDPTVVTTSTNDY
HDVVVVDVEDDPDEMAV

[0290] Modifications may be made to the foregoing without departing from the basic aspects of the invention. Although the invention has been described in substantial detail with reference to one or more specific embodiments, those of skill in the art will recognize that changes may be made to the embodiments specifically disclosed in this application, yet these modifications and improvements are within the scope and spirit of the invention, as set forth in the aspects which follow. All publications or patent documents cited in this specification are incorporated herein by reference as if each such publication or document was specifically and individually indicated to be incorporated herein by reference.

[0291] Citation of the above publications or documents is not intended as an admission that any of the foregoing is pertinent prior art, nor does it constitute any admission as to the contents or date of these publications or documents. U.S. patents and other publications referenced herein are hereby incorporated by reference.

What is claimed is:

1. A method for identifying a subject at risk of osteoarthritis, which comprises detecting the presence or absence of one or more polymorphic variations associated with osteoarthritis in a nucleic acid sample from a subject, wherein the one or more polymorphic variations are detected in a nucleotide sequence selected from the group consisting of:

- (a) a nucleotide sequence in SEQ ID NO: 1-17;
- (b) a nucleotide sequence which encodes a polypeptide encoded by a nucleotide sequence in SEQ ID NO: 1-17;
- (c) a nucleotide sequence which encodes a polypeptide that is 90% or more identical to the amino acid sequence encoded by a nucleotide sequence in SEQ ID NO: 1-17;
- (d) a fragment of a nucleotide sequence of (a), (b), or (c);

whereby the presence of the polymorphic variation is indicative of the subject being at risk of osteoarthritis.

2. The method of claim 1, which further comprises obtaining the nucleic acid sample from the subject.

3. The method of claim 1, wherein the one or more polymorphic variations are detected within a region spanning chromosome positions 21233000 to 21243000 in human genomic DNA.

4. The method of claim 1, wherein the one or more polymorphic variations are detected at one or more positions in SEQ ID NO: 1 selected from the group consisting of 238, 294, 295, 347, 1425, 4891, 5087, 7041, 7121, 7219, 7443, 7485, 10939, 11367, 11571, 11839, 12551, 12646, 13469, 14913, 15205, 15246, 15695, 17473, 17610, 17828, 18130, 18281, 18623, 18890, 21561, 23100, 23872, 24581, 24582, 24983, 27540, 30846, 31415, 31453, 31899, 37000, 38681, 39287, 42951, 45648, 46222, 46687, 47020, 47593, 48513, 49723, 49986, 53018, 53296, 53547, 53899, 53916, 53933, 54305, 55327, 55895, 56143, 56640, 58486, 59576, 63048, 64008, 64018, 64859, 65995, 66905, 67183, 67942, 68101, 68521, 68664, 68988, 69178, 72143, 74183, 74312, 74407, 75518, 76153, 77398, 77615, 79092, 80000, 80125, 80595, 81061, 81151, 81918, 83072, 83137, 83235, 83263, 83279, 83280, 83533, 86856, 87186, 87189, 87727, 87978, 89129, 89556, 89702, 90233, 93060, 94779, 95367, 95844, 95942, 96884, 96938, 97627, 97777, 97871, 98746 and 99663.

5. The method of claim 1, wherein the one or more polymorphic variations are detected at one or more positions in SEQ ID NO: 1 selected from the group consisting of 7219, 7485, 11839, 31899, 37000, 48513, 49986, 56640, 74407, 77398, 93060 and 97627.

6. The method of claim 1, wherein the one or more polymorphic variations are detected within a region spanning chromosome positions 102456500 to 102471500 in human genomic DNA.

7. The method of claim 1, wherein the one or more polymorphic variations are detected at one or more positions in SEQ ID NO: 2 selected from the group consisting of 225, 509, 860, 874, 939, 1483, 1798, 2189, 2215, 2282, 2340, 2963, 3369, 3481, 3564, 3653, 4860, 4941, 4975, 5321, 5346, 5541, 5633, 6007, 6317, 6378, 6382, 6426, 6479, 6641, 6703, 6705, 7963, 8525, 8526, 8598, 8624, 8883, 8980, 13578, 16135, 16141, 16642, 16931, 17004, 17009, 17010, 18713, 18853, 20783, 21335, 22180, 22268, 22285, 25378, 25906, 26015, 26475, 26798, 27042, 27649, 27827, 27873, 28122, 28202, 28232, 28240, 29546, 29748, 30054, 30646, 31149, 36912, 36936, 37184, 39064, 39343, 40868, 40917, 41113, 47343, 47806, 47911, 48009, 48621, 49245, 49247, 49299, 49302, 49514, 49626, 49791, 50010, 50294, 51482, 51556, 51855, 51956, 52155, 52448, 52458, 52511, 52607, 54049, 54224, 54567, 55052, 55857, 55941, 56120, 56349, 56727, 57232, 58806, 61181, 63808, 64526, 64865, 64928, 64966, 65080, 65690, 66228, 66982, 72511, 74170, 74264, 74333, 74502, 74741, 75321, 82558, 85366, 85469, 86485, 87687, 89463, 89660, 95718 and 95821.

8. The method of claim 1, wherein the one or more polymorphic variations are detected at one or more positions in SEQ ID NO: 2 selected from the group consisting of 2215, 3369, 16642, 20783, 52155, 55052, 55941, 74333, 74741, 85366, 85469, 87687, 89660 and 95718.

9. The method of claim 1, wherein the one or more polymorphic variations are detected within a region spanning chromosome positions 175647734 to 175655734 in human genomic DNA.

10. The method of claim 1, wherein the one or more polymorphic variations are detected at one or more positions in SEQ ID NO: 3 selected from the group consisting of 209, 5908, 7460, 7733, 7855, 7904, 8869, 9480, 13820, 15152, 17713, 17804, 18220, 19083, 19123, 19605, 20247, 20592, 21907, 23273, 23299, 23623, 23669, 23844, 24190, 24486, 24896, 25118, 30551, 30844, 30900, 30942, 31699, 32081, 35078, 36196, 36541, 38356, 45578, 49634, 49774, 51119, 51181, 51652, 54467, 55762, 55999, 57865, 66613, 68377, 69754, 72859, 76512, 76717, 77722, 80998, 82033, 89658, 89960, 94155 and 95679.

11. The method of claim 1, wherein the one or more polymorphic variations are detected at one or more positions in SEQ ID NO: 3 selected from the group consisting of 19083, 30900, 38356, 76512 and 94155.

12. The method of claim 1, wherein the one or more polymorphic variations are detected within a region spanning chromosome positions 105595000 to 105615000 in human genomic DNA.

13. The method of claim 1, wherein the one or more polymorphic variations are detected at one or more positions in SEQ ID NO: 4 selected from the group consisting of 241, 801, 899, 2091, 2290, 2440, 4959, 7914, 7969, 7972, 10831, 12399, 13841, 14461, 14680, 16808, 18231, 18394, 18505, 18684, 19257, 20263, 20656, 21499, 21563, 21612, 21834, 22406, 22408, 22685, 23303, 23306, 25139, 25211, 25364, 25381, 25414, 25835, 26214, 27224, 27526, 27934, 28550, 29015, 29879, 29979, 30030, 30585, 31753, 31934, 33227, 33228, 35172, 36901, 36921, 36932, 37061, 37570, 38745, 38970, 39725, 40070, 40460, 41470, 41562, 41956, 42047, 42280, 42358, 42629, 43075, 43387, 43393, 43438, 44115, 44537, 45642, 46629, 47496, 47515, 48329, 48862, 48908, 49038, 49080, 50204, 50404, 50426, 50531, 50840, 50964, 50971, 51378, 52610, 53906, 53951, 54111, 54149, 55563, 55999, 58415, 58961, 60447, 61377, 61528, 61606, 62140, 62461, 63826, 64950, 65076, 66121, 66406, 67051, 68860, 69014, 70796, 72325, 73414, 75258, 76347, 76839, 77358, 77822, 77946, 80002, 80024, 80285, 80397, 82075, 82153, 83981, 84184, 85089, 85288, 85330, 85581, 85642, 86433, 86904, 88391, 89042, 90828, 92676, 92881, 94227, 94585, 94616, 94712, 94738, 95253, 95522, 95869 and 97856.

14. The method of claim 1, wherein the one or more polymorphic variations are detected at one or more positions in SEQ ID NO: 4 selected from the group consisting of 25414, 25835, 38970, 41470, 44115, 47496, 49038, 50204, 50840, 50964, 50971, 53906, 54149, 58415, 70796, 72325, 75258, 77822, 80002, 85288, 85581, 86904, 90828, 94616, 94712, 95869 and 97856.

15. The method of claim 1, wherein the one or more polymorphic variations are detected within a region spanning chromosome positions 71957600 to 71962600 in human genomic DNA.

16. The method of claim 1, wherein the one or more polymorphic variations are detected at one or more positions in SEQ ID NO: 5 selected from the group consisting of 213, 249, 1824, 2057, 2306, 2869, 3976, 4288, 4290, 4434, 5298, 5467, 8486, 8487, 8831, 9036, 9058, 9131, 9732, 9862, 10191, 10270, 16167, 17620, 17751, 17764, 17787, 19401, 21021, 21902, 22173, 22416, 22653, 24945, 25011,

28563, 48574, 48710, 48880, 50194, 56343, 56455, 56729, 56759, 56895, 57036, 57702, 62515, 62629, 63501, 63547, 64876, 65073, 67149, 67549, 71660, 71906 and 71911.

17. The method of claim 1, wherein a polymorphic variation is detected at position 65073 in SEQ ID NO: 5.

18. The method of claim 1, wherein the one or more polymorphic variations are detected within a region spanning chromosome positions 76221000 to 76226000 in human genomic DNA.

19. The method of claim 1, wherein the one or more polymorphic variations are detected at one or more positions in SEQ ID NO: 6 selected from the group consisting of 205, 866, 4212, 5934, 11486, 16969, 22509, 22796, 28097, 28626, 28853, 28873, 30155, 30827, 31956, 32404, 32944, 35205, 35227, 35781, 41052, 45051, 46039, 47276, 47678, 47716, 51014, 54408, 54596, 56853, 61851, 62016, 62461, 68257, 69793, 73976, 73999, 74053, 75315, 75729, 76466, 77216, 77217, 79239, 80825, 81060, 81097, 81426, 84787, 84896, 85165, 86502, 86753, 86941, 88787 and 95598.

20. The method of claim 1, wherein the one or more polymorphic variations are detected at one or more positions in SEQ ID NO: 6 selected from the group consisting of 47716 and 69793.

21. The method of claim 1, wherein the one or more polymorphic variations are detected at one or more positions in linkage disequilibrium with one or more positions in claim 4, 7, 10, 13, 16 or 19.

22. The method of claim 1, wherein detecting the presence or absence of the one or more polymorphic variations comprises:

hybridizing an oligonucleotide to the nucleic acid sample, wherein the oligonucleotide is complementary to a nucleotide sequence in the nucleic acid and hybridizes to a region adjacent to the polymorphic variation;

extending the oligonucleotide in the presence of one or more nucleotides, yielding extension products; and

detecting the presence or absence of a polymorphic variation in the extension products.

23. The method of claim 1, wherein the subject is a human.

24. The method of claim 23, wherein the subject is a human female.

25. The method of claim 23, wherein the subject is a human male.

26. A method for identifying a polymorphic variation associated with osteoarthritis proximal to an incident polymorphic variation associated with osteoarthritis, which comprises:

identifying a polymorphic variation proximal to the incident polymorphic variation associated with osteoarthritis, wherein the polymorphic variation is detected in a nucleotide sequence selected from the group consisting of:

- (a) a nucleotide sequence in SEQ ID NO: 1-17;
- (b) a nucleotide sequence which encodes a polypeptide encoded by a nucleotide sequence in SEQ ID NO: 1-17;
- (c) a nucleotide sequence which encodes a polypeptide that is 90% or more identical to the amino acid sequence encoded by a nucleotide sequence in SEQ ID NO: 1-17;
- (d) a fragment of a nucleotide sequence of (a), (b), or (c) comprising a polymorphic variation;

determining the presence or absence of an association of the proximal polymorphic variant with osteoarthritis.

27. The method of claim 26, wherein the incident polymorphic variation is at one or more positions in claim 4, 7, 10, 13, 16 or 19.

28. The method of claim 26, wherein the proximal polymorphic variation is within a region between about 5 kb 5' of the incident polymorphic variation and about 5 kb 3' of the incident polymorphic variation.

29. The method of claim 26, which further comprises determining whether the proximal polymorphic variation is in linkage disequilibrium with the incident polymorphic variation.

30. The method of claim 26, which further comprises identifying a second polymorphic variation proximal to the identified proximal polymorphic variation associated with osteoarthritis and determining if the second proximal polymorphic variation is associated with osteoarthritis.

31. The method of claim 30, wherein the second proximal polymorphic variant is within a region between about 5 kb 5' of the incident polymorphic variation and about 5 kb 3' of the proximal polymorphic variation associated with osteoarthritis.

32. An isolated nucleic acid comprising a nucleotide sequence selected from the group consisting of:

- (a) a nucleotide sequence in SEQ ID NO: 1-17;
- (b) a nucleotide sequence which encodes a polypeptide encoded by a nucleotide sequence in SEQ ID NO: 1-17;
- (c) a nucleotide sequence which encodes a polypeptide that is 90% or more identical to the amino acid sequence encoded by a nucleotide sequence in SEQ ID NO: 1-17;
- (d) a fragment of a nucleotide sequence of (a), (b), or (c) comprising a polymorphic variation; and
- (e) a nucleotide sequence complementary to the nucleotide sequences of (a), (b), (c), or (d);

wherein the nucleotide sequence comprises a polymorphic variation associated with osteoarthritis selected from the group consisting of in SEQ ID NO: 1 an adenine at position 7219, a guanine at position 7485, an adenine at position 11839, a thymine at position 31899, an adenine at position 37000, a cytosine at position 48513, a guanine at position 49986, a guanine at position 56640, a cytosine at position 74407, a guanine at position 77398, an adenine at position 93060 and an adenine at position 97627; in SEQ ID NO: 2 an adenine at position 2215, a deletion at position 3369, a deletion at position 16642, a cytosine at position 20783, a cytosine at position 52155, a cytosine at position 55052, a cytosine at position 55941, a thymine at position 74333, an adenine at position 74741, a deletion at position 85366, a thymine at position 85469, a thymine at position 87687, an adenine at position 89660 and a cytosine at position 95718; in SEQ ID NO: 3 a thymine at position 19083, a guanine at position 30900, an adenine at position 38356, an adenine at position 76512 and an adenine at position 94155; in SEQ ID NO: 4 an adenine at position 25414, a cytosine at position 25835, an adenine at position 38970, an adenine at position 41470, an adenine at position 44115, a guanine at position 47496, a cytosine at position 49038, an adenine at position 50204, a thymine at position 50840, a cytosine at position 50964, a cytosine at position 50971, an adenine at position 53906, a guanine at position 54149, a guanine at position 58415, a thymine at position 70796, a guanine at position 72325, a cytosine at position 75258, an adenine at position 77822, an adenine at position 80002, an adenine at position 85288, an adenine at position 85581, a guanine at position 86904, a guanine at position 90828, an adenine thymine adenine adenine sequence at position 94616, a cytosine at position 94712, a guanine at position 95869 and a cytosine at position 97856; a guanine at position 65073 in SEQ ID NO: 5; and an adenine at position 47716 and a thymine at position 69793 in SEQ ID NO: 6.

33. An oligonucleotide comprising a nucleotide sequence complementary to a portion of the nucleotide sequence of (a), (b), (c), or (d) in claim 32, wherein the 3' end of the oligonucleotide is adjacent to a polymorphic variation associated with osteoarthritis.

34. A microarray comprising an isolated nucleic acid of claim 32 linked to a solid support.

35. An isolated polypeptide encoded by the isolated nucleic acid sequence of claim 32.

36. A method for identifying a candidate therapeutic for treating osteoarthritis, which comprises:

(a) introducing a test molecule to a system which comprises a nucleic acid comprising a nucleotide sequence selected from the group consisting of:

(i) a nucleotide sequence in SEQ ID NO: 1-17;

(ii) a nucleotide sequence which encodes a polypeptide encoded by a nucleotide sequence in SEQ ID NO: 1-17;

(iii) a nucleotide sequence which encodes a polypeptide that is 90% or more identical to the amino acid sequence encoded by a nucleotide sequence in SEQ ID NO: 1-17;

(iv) a fragment of a nucleotide sequence of (a), (b), or (c); or

introducing a test molecule to a system which comprises a protein encoded by a nucleotide sequence of (i), (ii), (iii), or (iv); and

(b) determining the presence or absence of an interaction between the test molecule and the nucleic acid or protein,

whereby the presence of an interaction between the test molecule and the nucleic acid or protein identifies the test molecule as a candidate therapeutic for treating osteoarthritis.

37. The method of claim 36, wherein the system is an animal.

38. The method of claim 36, wherein the system is a cell.

39. The method of claim 36, wherein the nucleotide sequence comprises one or more polymorphic variations associated with osteoarthritis.

40. The method of claim 39, wherein the one or more polymorphic variations associated with osteoarthritis are at one or more positions in claim 4, 7, 10, 13, 16 or 19.

41. A method for treating osteoarthritis in a subject, which comprises contacting one or more cells of a subject in need thereof with a nucleic acid, wherein the nucleic acid comprises a nucleotide sequence selected from the group consisting of:

- (a) a nucleotide sequence in SEQ ID NO: 1-17;
 - (b) a nucleotide sequence which encodes a polypeptide encoded by a nucleotide sequence in SEQ ID NO: 1-17;
 - (c) a nucleotide sequence which encodes a polypeptide that is 90% or more identical to the amino acid sequence encoded by a nucleotide sequence in SEQ ID NO: 1-17;
 - (d) a fragment of a nucleotide sequence of (a), (b), or (c); and
 - (e) a nucleotide sequence complementary to the nucleotide sequences of (a), (b), (c), or (d);
- whereby contacting the one or more cells of the subject with the nucleic acid treats the osteoarthritis in the subject.

42. The method of claim 41, wherein the nucleic acid is RNA or PNA.

43. The method of claim 42, wherein the nucleic acid is duplex RNA.

44. A method for treating osteoarthritis in a subject, which comprises contacting one or more cells of a subject in need thereof with a protein, wherein the protein is encoded by a nucleotide sequence which comprises a polynucleotide sequence selected from the group consisting of:

- (a) a nucleotide sequence in SEQ ID NO: 1-17;
 - (b) a nucleotide sequence which encodes a polypeptide encoded by a nucleotide sequence in SEQ ID NO: 1-17;
 - (c) a nucleotide sequence which encodes a polypeptide that is 90% or more identical to the amino acid sequence encoded by a nucleotide sequence in SEQ ID NO: 1-17;
 - (d) a fragment of a nucleotide sequence of (a), (b), or (c);
- whereby contacting the one or more cells of the subject with the protein treats the osteoarthritis in the subject.

45. A method for treating osteoarthritis in a subject, which comprises:
detecting the presence or absence of one or more polymorphic variations associated with osteoarthritis in a nucleic acid sample from a subject, wherein the one or more polymorphic variation are detected in a nucleotide sequence selected from the group consisting of:

- (a) a nucleotide sequence in SEQ ID NO: 1-17;

(b) a nucleotide sequence which encodes a polypeptide encoded by a nucleotide sequence in SEQ ID NO: 1-17;

(c) a nucleotide sequence which encodes a polypeptide that is 90% or more identical to the amino acid sequence encoded by a nucleotide sequence in SEQ ID NO: 1-17;

(d) a fragment of a nucleotide sequence of (a), (b), or (c) comprising a polymorphic variation; and

administering an osteoarthritis treatment to a subject in need thereof based upon the presence or absence of the one or more polymorphic variations in the nucleic acid sample.

46. The method of claim 45, wherein the one or more polymorphic variations are detected at one or more positions in claim 4, 7, 10, 13, 16 or 19.

47. The method of claim 45, wherein the treatment is selected from the group consisting of administering a corticosteroid, a nonsteroidal anti-inflammatory drug (NSAID), a cyclooxygenase-2 (COX-2) inhibitor, an antibody, a glucocorticoid, hyaluronic acid, chondroitin sulfate, glucosamine or acetaminophen; prescribing a heat/cold regimen or a joint protection regimen; performing joint surgery; prescribing a weight control regimen; and combinations of the foregoing.

48. A method for detecting or preventing osteoarthritis in a subject, which comprises:
detecting the presence or absence of one or more polymorphic variations associated with osteoarthritis in a nucleic acid sample from a subject, wherein the polymorphic variation is detected in a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence in SEQ ID NO: 1-17;

(b) a nucleotide sequence which encodes a polypeptide encoded by a nucleotide sequence in SEQ ID NO: 1-17;

(c) a nucleotide sequence which encodes a polypeptide that is 90% or more identical to the amino acid sequence encoded by a nucleotide sequence in SEQ ID NO: 1-17;

(d) a fragment of a nucleotide sequence of (a), (b), or (c) comprising a polymorphic variation; and

administering an osteoarthritis prevention or detection procedure to a subject in need thereof based upon the presence or absence of the one or more polymorphic variations in the nucleic acid sample.

49. The method of claim 48, wherein the one or more polymorphic variations are detected at one or more positions in claim 4, 7, 10, 13, 16 or 19.

50. The method of claim 48, wherein the osteoarthritis prevention is selected from the group consisting of administering a corticosteroid, a nonsteroidal anti-inflammatory drug (NSAID), a cyclooxygenase-2 (COX-2) inhibitor, an antibody, a glucocorticoid, hyaluronic acid, chondroitin sulfate, glucosamine or acetaminophen; prescribing a heat/cold regimen or a joint protection regimen; performing joint surgery; prescribing a weight control regimen; and combinations of the foregoing.

51. A method of targeting information for preventing or treating osteoarthritis to a subject in need thereof, which comprises:

detecting the presence or absence of one or more polymorphic variations associated with osteoarthritis in a nucleic acid sample from a subject, wherein the polymorphic variation is detected in a nucleotide sequence selected from the group consisting of:

- (a) a nucleotide sequence in SEQ ID NO: 1-17;
- (b) a nucleotide sequence which encodes a polypeptide encoded by a nucleotide sequence in SEQ ID NO: 1-17;
- (c) a nucleotide sequence which encodes a polypeptide that is 90% or more identical to the amino acid sequence encoded by a nucleotide sequence in SEQ ID NO: 1-17;
- (d) a fragment of a nucleotide sequence of (a), (b), or (c) comprising a polymorphic variation; and

directing information for preventing or treating osteoarthritis to a subject in need thereof based upon the presence or absence of the one or more polymorphic variations in the nucleic acid sample.

52. The method of claim 51, wherein the one or more polymorphic variations are detected at one or more positions in claim 4, 7, 10, 13, 16 or 19.

53. A composition comprising a cell from a subject having osteoarthritis or at risk of osteoarthritis and an antibody that specifically binds to a protein, polypeptide or peptide encoded by a nucleotide sequence identical to or 90% or more identical to a nucleotide sequence in SEQ ID NO: 1-17.

54. The composition of claim 41, wherein the antibody specifically binds to an epitope comprising a threonine at position 98 in a *APOB* polypeptide.

55. A composition comprising a cell from a subject having osteoarthritis or at risk of osteoarthritis and a RNA, DNA, PNA or ribozyme molecule comprising a nucleotide sequence identical to or 90% or more identical to a portion of a nucleotide sequence in SEQ ID NO: 1-17.

56. The composition of claim 55, wherein the RNA molecule is a short inhibitory RNA molecule.

Abstract of the Disclosure

Provided herein are methods for identifying a risk of osteoarthritis in a subject, reagents and kits for carrying out the methods, methods for identifying candidate therapeutics for treating osteoarthritis, and therapeutic and preventative methods applicable to osteoarthritis. These embodiments are based upon an analysis of polymorphic variations in nucleotide sequences within the human genome.

FIGURE 1A

APOB - FEMALE P-VALUES

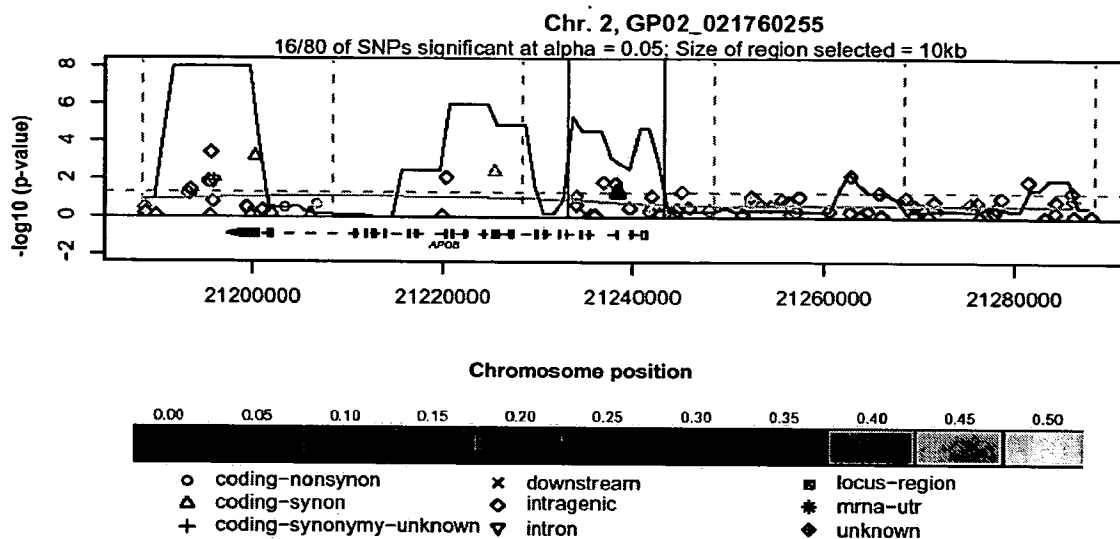


FIGURE 1B

IL1RL2 - DISCOVERY P-VALUES (female only)

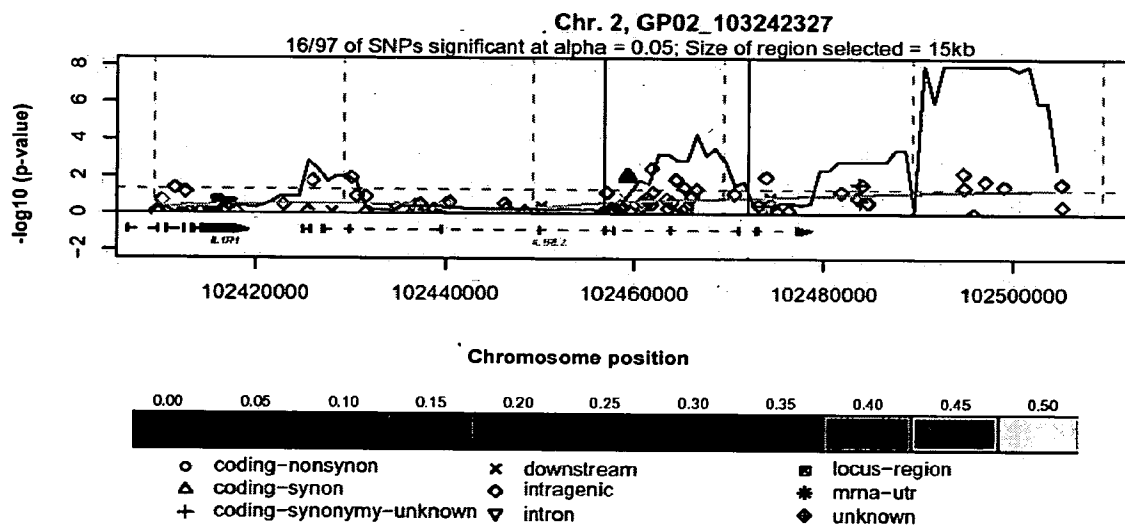


FIGURE 1C

WASPIP – DISCOVERY P-VALUES (female only)

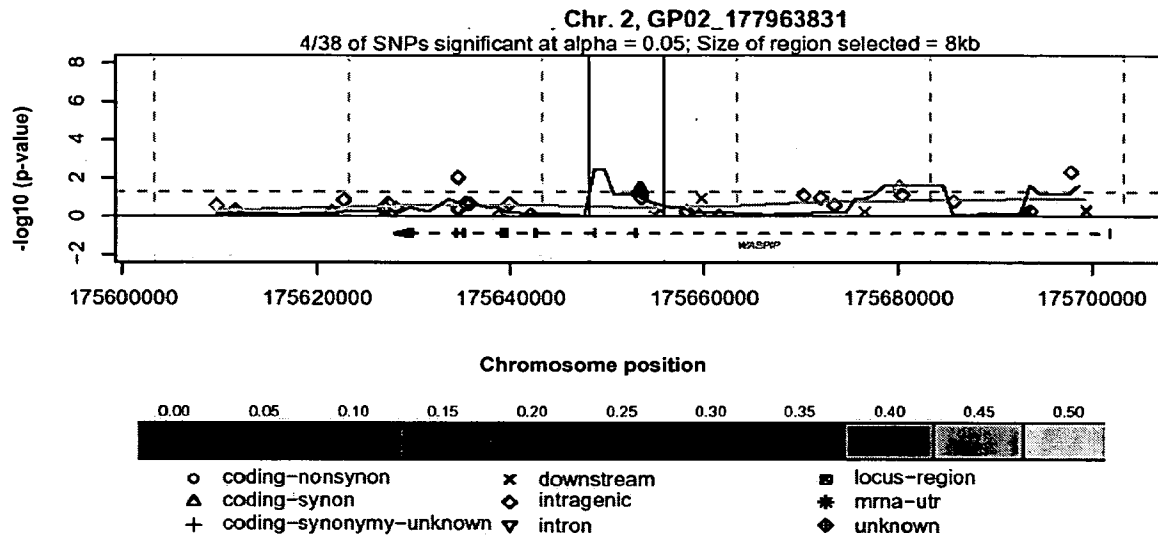


FIGURE 1D

BVES – DISCOVERY P-VALUES (female only)

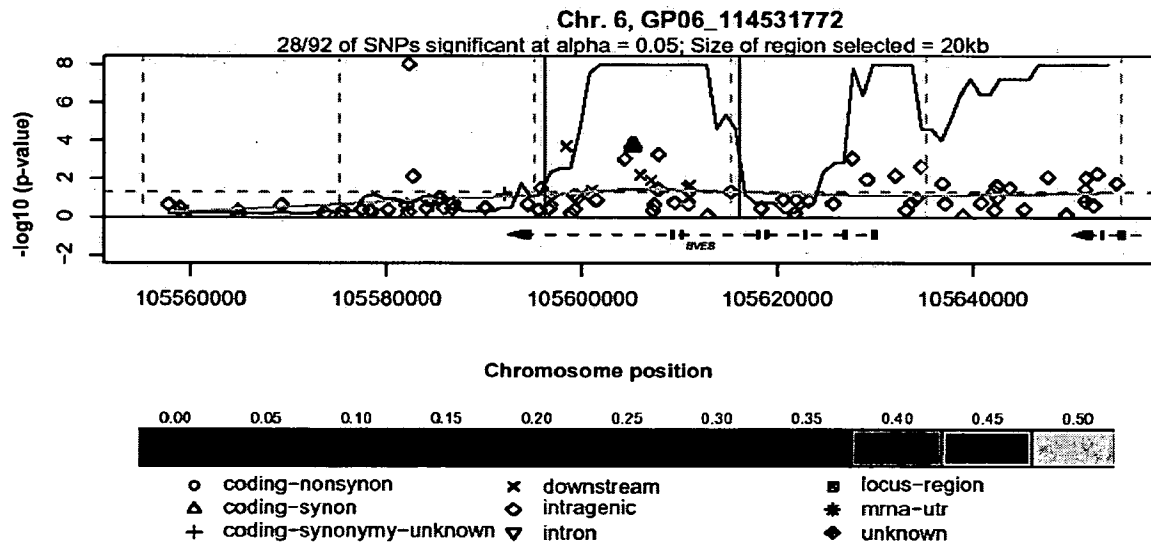


FIGURE 1E

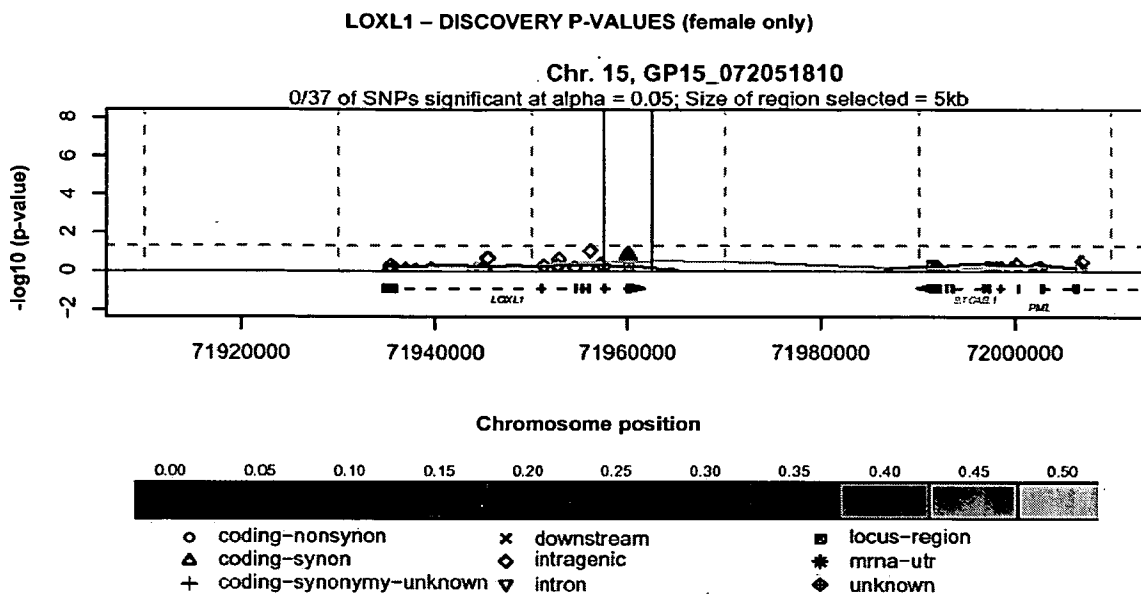
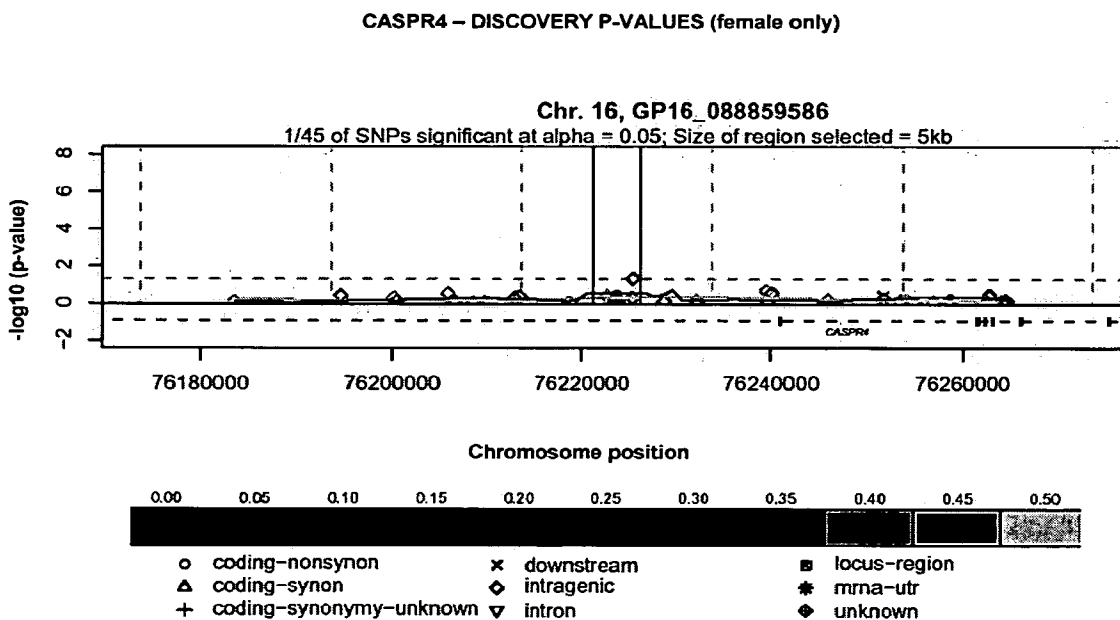


FIGURE 1F



Application Data Sheet

Application Information

Application Type::	Provisional
Subject Matter::	Utility
Suggested Group Art Unit::	Not Yet Assigned
CD-ROM or CD-R?::	None
Sequence submission?::	None
Computer Readable Form (CRF)?::	No
Title::	METHODS FOR IDENTIFYING RISK OF OSTEOARTHRITIS AND TREATMENTS THEREOF
Attorney Docket Number::	524593008800
Request for Early Publication?::	No
Request for Non-Publication?::	No
Total Drawing Sheets?::	3
Small Entity?::	Yes
Petition included?::	No
Secrecy Order in Parent Appl.?::	No

Applicant Information

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Country of Residence:: US
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